# SCIENCE

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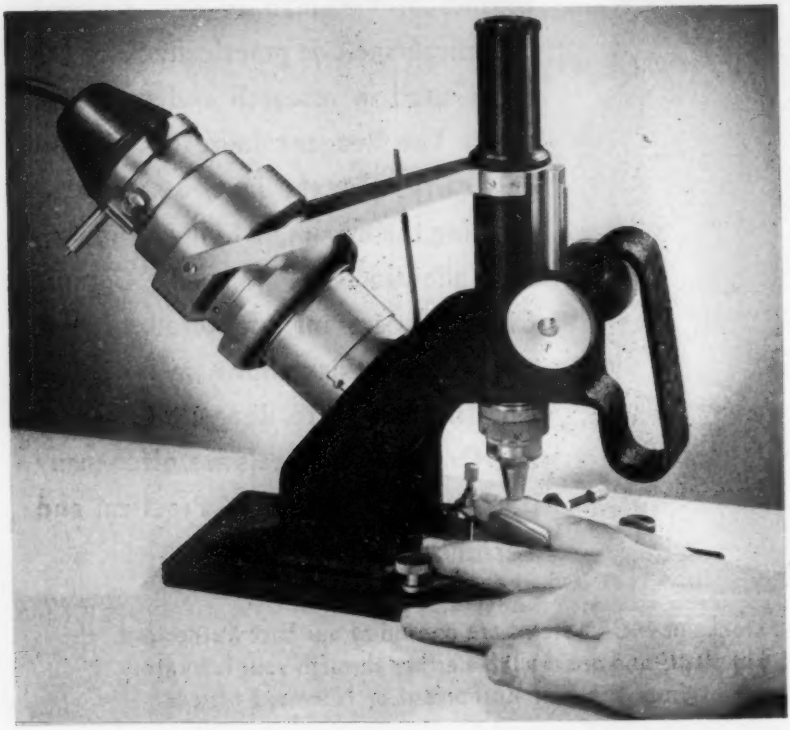

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# The 14th International Congress of Zoology

AT the 13th International Congress of Zoology it was decided to accept an invitation from the Danish Government to have the next congress in Copenhagen, and at the same time Professor R. Spaerck of the University of Copenhagen was elected as the president.

Accordingly, the 14th Congress will take place in Copenhagen from the 5th to the 12th of August, 1953. His Majesty the King of Denmark is the Patron of the Congress. Professor Niels Bohr is one of the members of the Honorary Committee. Professor Spaerck is leading the Organizing Committee, of which Dr. Anton Fr. Bruun is the Secretary General. So far about 400 zoologists outside Denmark have registered as members, besides 125 associate members. U.S.A., Great Britain, and Germany will each be represented by 50 or more, next comes France with 40, Sweden with 26, and Italy with 21. A Russian delegation of 10 has been announced. Altogether forty-three countries or territories from all over the world will be represented.

Before the Congress the International Union of Biological Sciences has arranged a colloquium on Nomenclature, to open on July 29, and organized by Mr. Francis Hemming. Another I.U.B.S. colloquium, on Zoogeographical Problems of the Deep Sea Fauna, will be held during the Congress, organized by Professor R. Spaerck, Copenhagen. To this Professor S. Ekman (Uppsala), Professor L. Fage (Paris), Professor Carl Hubbs and Professor Claude E. ZoBell (La Jolla), Mrs. Marion Grey (Chicago), Dr. J. D. H. Wiseman (London) and several others have been invited to present papers.

During the Congress 217 papers will be read; therefore about twenty sections have been formed to create opportunities for the specialists to meet and discuss their problems. Great attention will be paid to that most important tool of all zoologists, nomenclature. The next congress is expected to take place in 1958, the bicentennary of the *Systema Naturae*; it is to be hoped that decisions of the Copenhagen Congress will add materially in creating order in accordance with the spirit of Linnaeus.

The central position of comparative physiology is reflected by the great number of papers announced in that field, about 50. Ecology, however, is also very well represented, with 22 papers on marine ecology and 25 on terrestrial ecology. Morphogenesis is also one of the leading disciplines with 35 papers. The rest of the papers are fairly evenly spread over all aspects of zoology, except that such a specialty as nematology shows 16 papers. The number of participating zoologists and papers announced thus indicates that the Copenhagen Congress will meet with great interest, as is necessary for an international meeting.

After the Congress six excursions will show members typical biotopes of Denmark. Even at this late date the Secretariat of the Congress, Krystalgade 27, Copenhagen K, remains ready to receive applications for membership.

ANTON FR. BRUUN

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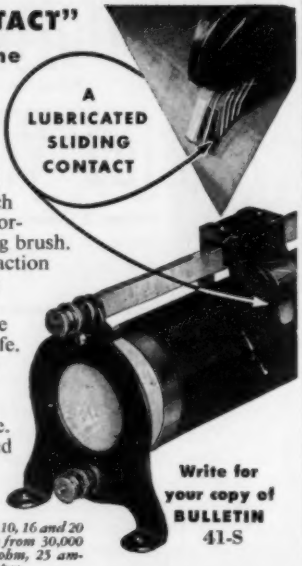


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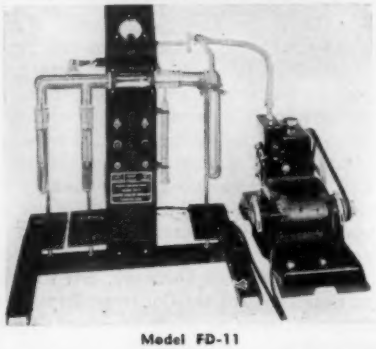

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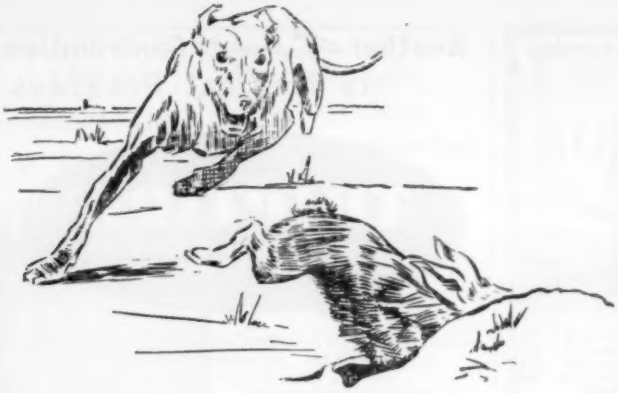

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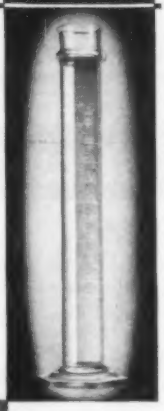

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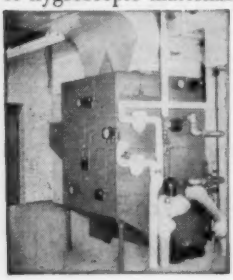

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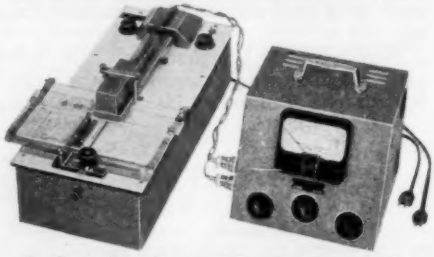

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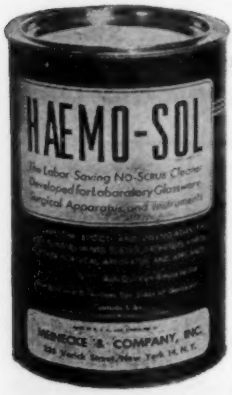


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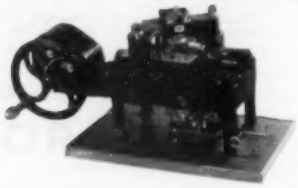

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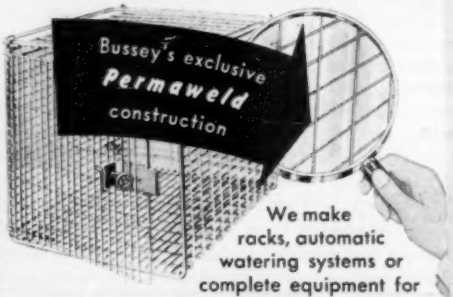


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The types of studies made at this Laboratory and elsewhere will illustrate some of the applications of the method to chemistry and physics. Even though the field is quite new, no attempt at a complete survey is intended, nor is it implied that all phenomena are completely understood. First, the nature of the phenomenon must be considered in more detail.

The absorption of radiofrequency energy is accompanied by transitions among closely spaced energy levels arising from the interaction of the atomic nuclei with their environment in a solid substance, and the frequency, $\nu$, is determined by $E = h\nu$, $E$ being the energy difference between the levels and $h$ Planck's constant. Hence an explanation of the phenomenon becomes a discussion of the nature of the energy levels involved. This in turn requires some elaboration of the nature of a quantity called the nuclear electric quadrupole moment and the particular features of the nuclear environment that interact with it to determine the levels.

*The Energy Levels.* Perhaps the most familiar of the nuclear moments are the magnetic (dipole) moment, $\mu$, and the mechanical moment (or intrinsic angular momentum), which is usually designated by a spin quantum number $I$. In general other magnetic and also electric moments can exist. The electric moments can be treated, for the purposes at hand, by considering the nucleus to be made up of a finitely sized assemblage of charges. One then finds that the electrostatic potential at a point outside the nucleus can be expressed as an infinite series whose successive terms correspond to potentials from certain idealized charge distributions known as higher and higher order electric moments. The first term of this expansion contains the nuclear charge which, in a sense, may be regarded as the simplest electric moment, a mono-pole with dimensions $eL^0$. The second term in the expansion of a general potential contains the electric dipole moment; however, the theory of nuclear structure implies that there should be no electric dipole moment in nuclei, and to date no experimental phenomenon has been found that requires the existence of such a moment. We are interested in the next term of this expansion which contains the nuclear electric quadrupole moment, $Q$. This moment has dimensions of $eL^2$, but it is customary to factor out the protonic charge $e$ and express $Q$ in cm$^2$. This electric moment is a measure of the departure from spherical symmetry of the nuclear charge distribution. The value for $Cl^{35}$, for example, is about $-0.08 \times 10^{-24}$ cm$^2$, the negative sign indicating that the nuclear charge distribution is flattened or oblate with respect to the nuclear-spin axis. A positive sign indicates a prolate distribution. $Q$ differs from zero only for nuclei with $I \geq 1$.

A quadrupolar nucleus placed in the neighborhood of other electric charges will give rise to an interaction energy, which in general depends on the relative orientation of the nuclear and extranuclear charge systems. This interaction is described in terms of the set of second partial derivatives with respect to coordinates, $q_{ij}; i,j = x,y,z$, of the electrical potential at the location of the nucleus (thus $q_{zz} = \partial^2 V/\partial z^2$, etc.). This interaction results in the quadrupolar nucleus taking a number of allowed orientations in the $q_{ij}$ system, and the energy for each is different. The number of quantum-allowed orientations is determined by the nuclear spin, two being possible for each of the stable chlorine isotopes with $I = 3/2$. The absorption of a quantum of radiofrequency energy by such a system induces a change in orientation from a lower energy configuration to a higher one. The set of second derivatives, which is commonly referred to as the electric field gradient, is a second order tensor quantity. With a proper choice of coordinate axes, the tensor may be represented by three orthogonal components, $|q_{zz}| > |q_{xx}| > |q_{yy}|$, and these conform to Laplace's equation, $q_{zz} + q_{xx} + q_{yy} = 0$. Frequently $q_{zz}$, the major component of the tensor, is abbreviated by $q$. The energy of the quadrupolar interaction is often expressed in terms of a parameter called the nuclear electric quadrupole coupling, $eqQ$, and this parameter is usually evaluated when the phenomenon is observed.

As a simple example, let us consider a quadrupolar nucleus aligned in the field gradient of a single (classical) electron with charge $-e$ at a distance $r$ from the nucleus. The electric potential at the nucleus is

[1] This work was performed for the Atomic Energy Commission.

$-e/r$ and $q_{zz}$ is $-2e/r^3$. The quadrupole coupling becomes $-2e^2Q/r^3$ and $q_{xx}=q_{yy}=e/r^3$. In this simple case the coupling is said to have axial symmetry. In many physical systems where more complex charge distributions are involved $q_{xx}$ and $q_{yy}$ may not be equal, the asymmetry frequently being denoted by a parameter $\eta=[q_{xx}-q_{yy}]/q_{zz}$.

The energy levels for axial symmetry, $\eta=0$, are given by $E_m=eqQ[3m^2-I(I+1)]/4I(2I-1)$ where $m$ may have values $I, I-1, \cdots, -I$. Since $m$ appears only as the square, all levels are twofold degenerate. For $I=3/2$, the case for all stable chlorine and bromine nuclei, $E_{\pm 1/2}=-eqQ/4$ and $E_{\pm 3/2}=eqQ/4$. With the selection rule $\Delta m=1$ the transition energy is given by $h\nu=|eqQ/2|$. The quadrupole coupling is often expressed in frequency units and for this case is just twice the observed transition frequency. Since the sign of the coupling is not determined, only its magnitude and the magnitude of $q$ are considered. The case of departure from axial symmetry is considered later. Properties of extranuclear charge distributions giving rise to significant quadrupole couplings are discussed in a later section.

*Experimental.* Although the mechanism for establishing the energy levels is electric in nature, the main process by which the nucleus absorbs radiofrequency energy in making a transition is magnetic. For this reason the sample is placed in the coil of a tuned circuit of a radiofrequency oscillator where it experiences oscillations in magnetic field. The oscillator is frequency modulated at, say, 100 cps. If the oscillator is now tuned to the frequency of a quadrupole resonance, the oscillator frequency will be swept back and forth through the resonance frequency 100 times a second. Each time the resonance line is crossed, energy is absorbed from the oscillator coil and the circuit is so arranged that the resulting signal can be taken from the circuit, amplified, and presented on the vertical plates of an oscillograph. The horizontal plates of the oscillograph are driven at 100 cps in synchronization with the frequency modulation. Figure 1 is a photograph of a quadrupole line observed in this manner. In this case the line is only a few kilocycles wide. Other electronic arrangements can be used, the principal extension of the technique being the use of a chart recorder and appropriate electronic circuits that allow the line to be seen with a higher signal-to-noise ratio.

*Resonance Lines for Chlorine.* Each of the stable chlorine isotopes possesses a quadrupole moment, and their resonance lines can be seen in many compounds. If the electrical environment of the nucleus were independent of isotopic substitution, it would follow from the energy level expression that the ratio of observed frequencies for the two stable chlorine isotopes in a given compound should be their quadrupole moment ratio. The ratios given in Table 1 do not vary outside of the accuracy of the frequency measurements. More recent data (*2*) of greater accuracy do, however, show a very small significant variation.

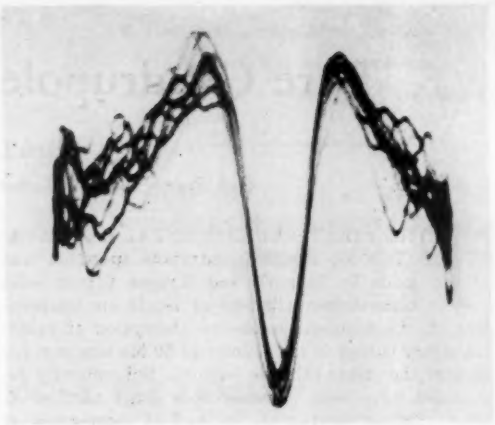

FIG. 1. The 38.254 Mc $Cl^{35}$ line in $CHCl_3$ at 77° K.

Nevertheless, to a very high precision $q$ is a property of the substance that is quite insensitive to isotopic substitution, and accurate quadrupole moment ratios can be determined. Resonance lines for $Cl^{35}$ are much stronger than those for $Cl^{37}$ owing, in part, to the three times greater natural abundance. Frequently just the $Cl^{35}$ lines are measured since the weaker $Cl^{37}$ values can be easily calculated.

Often two or more lines are seen for each chlorine isotope (Tables 1 and 2). A similar effect was first seen and explained by Dehmelt (*3*) for the iodine resonances in $SnI_4$. The number of lines corresponds to the number of nonequivalent halogen positions in the crystal lattice. Thus each atom experiences a particular $q$, the magnitude of which depends on the particular lattice position it occupies. The value of $q$ in these substances does not change greatly from one type lattice position to another as evidenced by the close spacing of the multiple lines. Fourteen weak lines were originally seen for $CCl_4$ (Table 2) and these were measured at 20° K. Later with a more sensitive spectrometer, one of the lines at 77° K was resolved into a doublet, which now gives a total of 15 lines. The crystal structure may be like that of $CBr_4$ which is reported (*4*) to be monoclinic and of large cell dimensions, apparently with 32 molecules per unit cell. It is likely that there is a sixteenth line in $CCl_4$ that has not yet been observed.

The frequencies of quadrupole lines shift with temperature changes, in most cases increasing with decreasing temperature. A theory based on a torsional oscillator model has been proposed (*5*) to explain the effect. Briefly the concept is that if there are rapid oscillations (compared to the transition frequency) in the charge distribution, then the effective value of $q$ is an average over the amplitude of these oscillations and is thus of smaller magnitude than that given by the same charge distribution at rest. Thus decreasing the temperature decreases the amplitude of oscillations over which $q$ is averaged, and a higher resonance

TABLE 1

MEASURED FREQUENCIES AND RATIOS FOR SOLID Cl COMPOUNDS AT 77° K

| Compound | $\nu(Cl^{35})$ Mc | $\nu(Cl^{37})$ Mc | $\nu(Cl^{35})/\nu(Cl^{37})$ |
|---|---|---|---|
| $SOCl_2$ | 32.0908 | 25.2935 | 1.26874 |
| | 31.8874 | 25.1331 | 1.26874 |
| $CH_2Cl_2$ | 35.9912 | 28.3673 | 1.26876 |
| $Cl_2$ | 54.2475 | 42.7544 | 1.26882 |
| $CHCl_3$ | 38.3081 | 30.1921 | 1.26881 |
| | 38.2537 | 30.1500 | 1.26878 |
| $POCl_3$ | 28.9835 | 22.8432 | 1.26880 |
| | 28.9378 | 22.8067 | 1.26883 |

TABLE 2

MEASURED FREQUENCIES (Mc) FOR $Cl^{35}$

| Compound | 77° K | 20° K | 4° K |
|---|---|---|---|
| $CCl_4$ | 40.465 | 40.732 | |
| | 40.521 | 40.794 | |
| | 40.540 | 40.800 | |
| | 40.549 | 40.826 | |
| | 40.576 | 40.831 | |
| | 40.587 | 40.888 | |
| | 40.607 | 40.895 | |
| | 40.639 | 40.918 | |
| | 40.643 | 40.965 | |
| | 40.655 | 40.972 | |
| | 40.696 | 41.022 | |
| | 40.721 | 41.045 | |
| | 40.782 | 41.109 | |
| | 40.797 | 41.139 | |
| | 40.817 | | |
| $CH_3Cl$ | 34.029 | 34.199 | 34.206 |
| $AsCl_3$ | 24.960 | 25.048 | 25.054 |
| | 25.058 | 25.158 | 25.167 |
| | 25.406 | 25.520 | 25.528 |
| $CH_2Cl_2$ | 35.991 | 36.233 | 36.243 |
| $CCl_3CCl_3$ | 40.761 | 40.885 | |
| | 40.714 | 40.823 | |
| | 40.685 | 40.798 | |
| | 40.551 | 40.652 | |

frequency results. At sufficiently low temperatures one would expect excited lattice vibrations to be essentially frozen out, and the resonance frequency would stop increasing with decreasing temperature. The measurements in Table 2 show a moderate frequency increase in cooling from 77° K to 20° K, while further cooling to 4° K gives very little additional increase. Dean and Pound (*6*) have reported higher temperature data on a number of substances. In some cases crystal phase transitions gave discontinuities in a frequency-temperature graph. One compound had four closely spaced lines below the discontinuity while two were present above. Usually the frequency shift at such discontinuities is small, being of the same order as the separations of multiple lines.

*Chemistry.* Nuclear quadrupole couplings have been determined in a number of gaseous substances by studying hyperfine structure of rotational transitions in the microwave region. Townes and Dailey (*7*) have discussed the origin of $q$ and have shown that it is almost entirely due to the electron distribution in the chemical bond that holds the atom containing a quadrupolar nucleus in the molecule. Their development is based on an atomic orbital model of chemical bonding, and the general features may be applied to solid systems.

Townes and Dailey point out that $q$ has a very large magnitude in many molecules. For example, in many chlorine compounds $q$ must be of the order of $10^{16}$ esu as determined from magnitudes of observed couplings. A (classical) electron near the nucleus, say at a distance of 1 A, would give a contribution to $q$ over an order of magnitude smaller than the observed $10^{16}$ esu. A reasonable way to account for such large field gradients is to make use of appropriate atomic orbitals. For example, atomic chlorine has a quadrupole coupling of about (−) 110 Mc from measurements by an atomic beam experiment (*8*). The closed electron shells have spherical symmetry and do not contribute to $q$. The outer shell, however, lacks a $p$ electron, and it is this departure from spherical symmetry that gives $q$ a large value. In this case a deficiency of a $p$ electron from a full shell may be regarded as similar to a surplus of a $p$ electron. It is the net unbalance in $p$ orbitals that matters. A $p$ electron is so effective because of its nonspherical angular distribution near the nucleus (penetrating orbital). Electrons described by $s$ type wave functions do not contribute to $q$ because of the spherical symmetry of $s$ states. The coupling found in solid chlorine at 20° K is only 0.7 per cent lower than the atomic beam value. This has been taken as an indication that the chemical bond in $Cl_2$ uses essentially a pure $p$ atomic orbital with very little if any $s$ hybridization. If the bonding orbitals could be better described by a mixture of $s$ and $p$ orbitals, there would be less *net unbalance* in the electron distribution about the chlorine nuclei and $q$ would be smaller.

If the chlorine atom in the example above were converted to an ion by adding an electron, it would have a noble gas configuration with no unbalance in the electron distribution about the nucleus, and $q$ should be zero. If, following Pauling (*9*), a bond to chlorine with partial ionic character is considered, the foregoing concepts lead usually to the correlation of reduced couplings with increased ionic character. Thus couplings found for many solid inorganic chlorine compounds where there is a moderate amount of ionic character ($BCl_3$, $AsCl_3$, $SnCl_4$, $SiCl_4$, $PCl_3$, etc.) generally are lower than the more covalently bonded organic chlorides ($CHCl_3$, $CCl_4$, etc.). An interesting example of a reversed type of ionic character is in ClF which has a coupling much higher than $Cl_2$. In this case there may be contributions from the resonant structure $Cl^+ F^-$, and the ionic character is in the direction of increasing the departure from spherical symmetry in the charge distribution about the chlorine nucleus. Finally, if a partial double bond is formed, the coupling will be lowered. For example, if an atom is bonded with essentially a sigma $p$ orbital, $q$ will have a large magnitude. If there is also partial double bond character through the use of pi $p$ orbitals, there

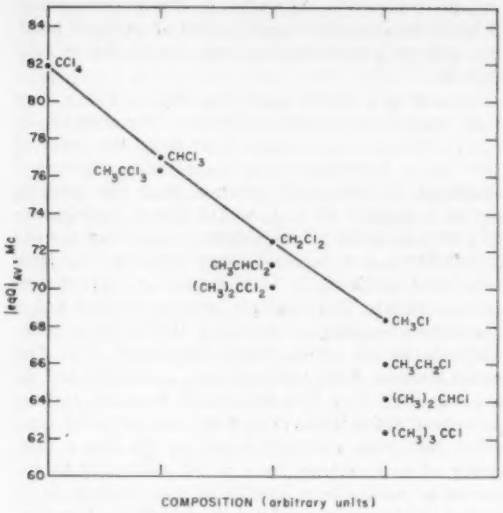


FIG. 2. $Cl^{35}$ quadrupole couplings in alkyl chlorides at 20° K.

will be some contributions to $q$ from this source. These contributions are properly represented by tensors. One effect of the pi contribution is to give a tensor element that subtracts from the magnitude of the major element ($q_{zz}$) of the sigma contribution. The net effect is a decreased coupling. A second effect of the pi contribution is to give asymmetry to the coupling, $\eta \neq 0$. This effect will be briefly mentioned later.

The chloroethanes (Fig. 2) show a coupling decrease each time a Cl atom is replaced by H. Replacement of H atoms by $CH_3$ groups results in further systematic coupling decreases. Here the coupling is taken as twice the observed transition frequency, the average frequency being used in cases where closely spaced multiple lines are seen. The coupling trend may be explained in terms of variations in the amount of ionic character in the bond. For example, the more chlorine atoms per molecule the less the ionic character in each C–Cl bond and the higher the coupling. The interpretation is consistent with the general chemical properties of the substances involved. Additional correlations have been made on several chloroethanes, and in each case the coupling trend may be reasonably explained by considering the electronegativities of the atoms involved and their effect on the C–Cl bond ionic character. The one carbon Freons have been correlated in a similar manner, but in this case the use of an additional parameter, partial double bond character, was used. Pauling (*9*) first proposed this concept ($F^-C=Cl^+$ resonant structures) to explain bond lengths in the Freons. Measurements have recently been made on several corresponding bromine and iodine compounds (*10*), and there is a striking parallelism in trend. A few of these include the coupling in $CH_2Br_2$ which is appropriately higher than $CH_3Br$ while $CH_3CH_2Br$ is lower. Moreover the value in $CH_3CH_2CH_2Br$ is very close to $CH_3CH_2Br$, very similar to the corresponding chlorine cases. Comparisons of solid values with microwave gas values show good agreement in several cases and marked departures, up to 10 per cent, in others. Although the solid values are for molecular crystals where the intermolecular interactions are presumably weak, the comparisons seem to suggest a change in the nature of the bond in going from the gaseous to the solid state. One of the large problems is to properly interpret the role of lattice effects. Townes and Dailey (*11*) have interpreted the quadrupole data on solid iodine in the light of its crystal structure. They conclude that in addition to the main molecular covalent bond, there are two weak intermolecular bonds to each atom.

*The Zeeman Effect.* Bersohn (*12*) has given a theoretical treatment of nuclear quadrupolar systems both in the absence and presence of a magnetic field. A magnetic field will remove the twofold degeneracy of the quadrupolar levels and in principle allow a larger number of lines (Zeeman components) to be observed. In order to illustrate the phenomenon a simple case will be considered. The energy levels and allowed transitions for chlorine ($I=3/2$) in the presence and absence of an applied magnetic field are indicated in Fig. 3. The magnitude of the magnetic splitting (spacing of Zeeman components) depends on the magnetic field strength, $H$, and the angle, $\theta$, between the direction of the magnetic field and $q_{zz}$. We will consider the coupling to have axial symmetry, $\eta=0$. For an arbitrary angle $\theta$ and for $I=3/2$, a single quadrupole line will split into four Zeeman components symmetrically displaced about the original frequency. For special values of $\theta$ some of the lines coincide. Only two lines are present for $\theta=90°$ and three lines for $\theta=54°44'$, the central line coinciding with the frequency of the unsplit quadrupole line. A powder sample gives a random spread in values for $\theta$, and the application of a magnetic field merely causes the line to broaden and, with high enough field, become undetectable.

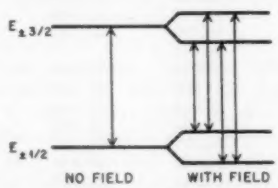


FIG. 3. The nuclear electric quadrupole energy levels for chlorine, with and without an applied magnetic field.

Let us consider the Zeeman effect in a single crystal of sodium chlorate (*10*). The crystal structure (*4*) is cubic with four molecules in the unit cell, and all chlorine atoms occupy equivalent lattice positions. In the absence of a magnetic field a single quadrupole resonance for $Cl^{35}$ appears at about 30 Mc. The four $ClO_3^-$ ions in the unit cell have a pyramidal configuration with a threefold axis of symmetry, and these

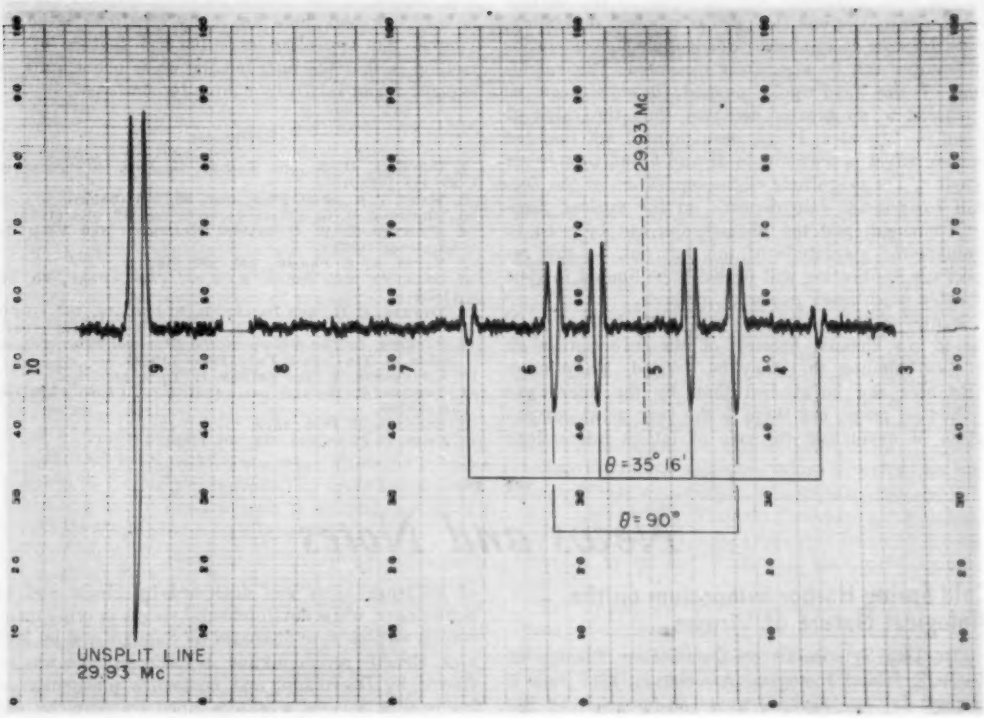


FIG. 4. Quadrupole resonance in a $NaClO_3$ single crystal without an applied magnetic field (left) and with a field of approximately 50 gauss applied along a twofold axis of the cube.

axes lie along the directions of the four body-diagonals of the cube. The direction of $q_{zz}$ for each lies along these symmetry axes, and from crystal symmetry $\eta = 0$. Thus each chlorate ion has its own field gradient system, equivalent except for direction, and the application of an external magnetic field will, in general, give a separate set of four Zeeman components for each of the four chlorine atoms. The nature of the splittings will depend on the orientation of the magnetic field with respect to the diagonals of the cube. If the field is oriented normal to a face of the cube, it makes an angle of 54°44′ with each of the diagonals, and all four sets of Zeeman components are superimposed. A total of three lines are present since this special angle gives a three line pattern.

Figure 4 is a recording of the Zeeman components for $Cl^{35}$ in sodium chlorate with a field of approximately 50 gauss oriented along a face diagonal (twofold axis) of the cube. The field direction makes an angle of 90° with two of the body diagonals giving two components and an angle of 35°16′ with the other two diagonals giving four more components. A recording is also shown for the unsplit quadrupole line. The second derivative of the absorption line is recorded.

*Asymmetry in the Field Gradient Tensor.* A measurement of $\eta$, the departure of the coupling from axial symmetry, often allows more to be understood about the chemical substance. In the previously mentioned study of the intermolecular bonding in solid iodine (*11*) $\eta$ was one of the important parameters. If the coupling is not axially symmetric a different expression must be used for the energy levels (*12*). The magnitude of $\eta$ can be evaluated directly from the pure quadrupole spectrum for all spin cases except $I = 3/2$. For example, the energy levels of the iodine nucleus ($I = 5/2$) with $\eta = 0$ give rise to two lines, one having exactly twice the frequency of the other. However, if $\eta \neq 0$ the ratio is not 2:1, and the actual ratio can be used to evaluate the asymmetry. Dean (*13*) has demonstrated that the asymmetry can also be evaluated for the $I = 3/2$ case by performing a Zeeman experiment. He observed the chlorine resonance in a single crystal of *p*-dichlorobenzene in the presence of a constant magnetic field. By carefully studying the line frequencies with different orientations of the crystal in the magnetic field he was able to locate the directions of the principal axes in the crystal and evaluate $q$ and $\eta$. He indicated that the asymmetry could be due to double bond character in the C–Cl bond.

Quadrupole spectroscopy is a field that has at-

tracted both physicists and chemists. It has certain areas of applications for nuclear moment determinations. In some cases nuclear quadrupole moment ratios of isotopes can be accurately measured. The magnitude of the quadrupole moment, however, can be evaluated to no greater accuracy than the value of $q$ can be estimated. It has been pointed out that the Zeeman effect is sensitive to H and $\theta$ and to a lesser extent $\eta$. The magnitude of the separation of the Zeeman components also depends on the nuclear magnetic moment, and the phenomenon can be used to evaluate the magnitude of this moment. In this respect the application will probably be limited in view of other much more powerful methods.

Studies on the nature of $q$ are particularly appealing to the chemist because of the information on chemical binding that may be derived. Many more substances can be studied than by the microwave method on gases, but there is the very great complication of evaluating the role of lattice interactions and motions in the lattice. In these respects the field is very new and there are many questions to be answered. An appealing experimental feature of the method is that the instrumentation is often quite inexpensive and relatively simple to construct.

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# *News and Notes*

## Cold Spring Harbor Symposium on the Biological Nature of Viruses

The 18th Symposium on Quantitative Biology of the Long Island Biological Association, held June 5 through 11, was devoted to a consideration of the biological nature of viruses, and of the interactions between viruses and their host cells. M. Delbruck opened the Symposium with an outline of the program, in which he distinguished and defined three states of viruses, the infective, vegetative, and provirus states. In the infective state the virus is extracellular, metabolically inert, resting between cycles of reproduction. It is extracellular virus which has been most extensively studied by physical, chemical, and immunological techniques, and which is best known because of its accessibility. It is however of less interest to biologists than the intracellular states in which the virus demonstrates some of the properties of a living organism. In the vegetative state, the virus is intracellular, virus reproduction occurs, genetic changes of the virus take place, and the host cell metabolism is so disorganized that the pathology of virus diseases is produced. In the provirus state, the virus coexists with its host cell in a symbiotic relationship in which the virus assumes the *de facto* role of a genetic unit of its host cell. The provirus protects its host cell against the attack of related viruses, profoundly affects the genetics of its host cell, and is a natural method for the storage and preservation of viruses.

Evidence for the existence of the vegetative state in bacteriophages was presented by Doermann, and the stages in its maturation to the infective state were discussed by Levinthal. The intracellular development of influenza virus was described by Henle and by Schlesinger, while developmental stages in other mammalian viruses were discussed by Sanders and by Melnick. Genetic recombination in bacteriophage was reviewed by Doermann, and analogous phenomena in mammalian viruses were described by Appleby and by Hirst, and a paper on this subject by Burnet was read by Fenner. The striking similarities between bacteriophages and animal viruses were made apparent, as well as the extraordinary difficulty of studying animal viruses by the usual techniques.

The properties of the provirus state in lysogenic bacteria were discussed by Bertani, including genetic interactions with related viruses and the protective effect of provirus against host cell destruction by related viruses. Bertani suggested that the prophage may be attached to some chromosome-like structure in the bacterial nucleus and so divides with it in cell duplication. Experiments on the inheritance of prophage lambda in crosses of *E. coli* K12, reported by Appleyard, were consistent with this hypothesis. The conditions involved in the transition from infective phage to prophage were discussed by Bertani and by Lieb. The physiological state of the host cell, the temperature, and the multiplicity of infection are important factors, as well as the genetic constitutions of host cell and virus. The transition from provirus to the vegetative state, the phenomena of induction, was described by Jacob, who suggested that the primary event is a local disturbance in the bacterium which dislodges the prophage from its locus on the bacterial chromosome so as to permit its uncontrolled multiplication. Evidence suggesting that genetic recombination in bacteria may be mediated by an agent analogous to a virus was presented by Hayes. This

agent controls the fertility of the bacterial cell and the direction of genetic transfer and possibly acts as a vector of genetic material.

The structure of the phage particle in the infective state was described from information derived from immunological methods by Lanni, from electron microscopy by Robley Williams and T. F. Anderson, and from interactions with the ionic environment by Lark and Adams. The phage particle is differentiated morphologically and immunologically into head and tail, and chemically into protein and nucleic acid. The phage particle becomes attached to the host cell by the tip of its tail, the protein portion being an apparatus for the introduction of nucleic acid into the host cell. The evidence presented by Hershey that nucleic acid alone may be the determinant of genetic specificity in viruses, as had previously been demonstrated for bacteria, has focused renewed interest on the structure of the nucleic acid molecule. Recent concepts of nucleic acid structure were presented by Watson and by Markham and were discussed in relationship to virus reproduction. The nucleic acid molecule seems complicated enough to account for all the specificity demanded by the geneticist.

The fate of the chemical components of the infecting virus particle, and the source of the raw materials needed for virus multiplication were discussed by Hershey and by Kozloff. The parent phage particle does contribute some material to the progeny, but the complexity of this material is still unknown. The bacterial contribution seems to consist mainly of structurally simple substances. The effects of virus infection on host cell metabolism were discussed by S. S. Cohen, who suggested various shifts in carbohydrate and pyrimidine syntheses to explain the observed results. The new base, hydroxymethylcytosine, discovered in phage T2 by Cohen and Wyatt, played a prominent role in these discussions.

The marked effect of virus reproduction on the properties of the infected host cell has always been evident to virologists. That the host cell may modify the properties of the infecting virus has not been so obvious. Several examples of temporary modifications of host range in phages produced by passage through certain host cells were discussed by Luria. The very important role that prophages may play in the adaptive modification of the typhoid typing phages was pointed out by E. S. Anderson. The mechanism of these induced modifications of viral properties is still obscure. The role of the host cell in reviving the infectivity of phages inactivated by ultraviolet irradiation was discussed by Bowen.

An important property of bacteriophages is the ability to transfer genetic characteristics from one bacterial strain to another. Zinder presented the hypothesis that genetically active fragments of host cell nucleic acid are built into the virus particle, which then serves as a vector for the transport of this material and for its introduction into a second bacterium. The similar process of bacterial transformation, in which bacterial nucleic acid is transferred from cell to cell without an obvious vector, was discussed by Hattie Alexander.

The final session of the symposium was devoted to striking developments in tissue culture methods for the study of mammalian viruses. Plating methods for the production of virus plaques with encephalitis and poliomyelitis viruses were described by Dulbecco, who has also used suspensions of mammalian cells for one-step growth and single-cell experiments. Syverton described pure cultures of single types of mammalian cells which can be subcultured indefinitely *in vitro* and which support growth of many strains of viruses. The development of these tissue culture techniques should greatly facilitate the investigation of the biological properties of mammalian viruses.

The study of viruses as organisms rather than as the agents of disease was envisaged by Burnet years ago, but suitable techniques were not then available. In recent years, however, the subject of virus research has changed from the extracellular, infective state to the intracellular, vegetative state, in which the virus plays its role as an organism and as a pathogen. It is to the credit of the National Foundation for Infantile Paralysis that they have supported much of the basic research which has made this change of emphasis possible.

The Symposium on Viruses owed much of its success to the interest and support of the National Foundation for Infantile Paralysis. The papers and discussions will be published as Vol. 18 of the *Symposia on Quantitative Biology* before the end of the year.

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*New York University, Bellevue Medical Center*

## Scientists in the News

**Fred Allison** has retired as Head of the Department of Physics and Dean of the Graduate School at Alabama Polytechnic Institute, Auburn.

**F. C. Bishopp,** since 1904 associated with the U.S. Department of Agriculture's Bureau of Entomology and Plant Quarantine, has resigned as Assistant Bureau Chief to accept a position with the Oscar Johnston Cotton Foundation as coordinator of all federal, state, and industry-sponsored research concerned with the control of the pink bollworm. His headquarters will be at Brownsville, Tex. He is succeeded in the Department of Agriculture by **Edward F. Knipling,** who joined the Bureau of Entomology and Plant Quarantine in 1930. Since 1946 Dr. Knipling has been leader in the Bureau's Division of Insects Affecting Man and Animals.

**Jonas J. Christensen,** staff member of the Institute of Agriculture at the University of Minnesota since 1920, has succeeded **Elvin C. Stakman** as Head of the University's Department of Plant Pathology and Botany. In 1929–30 Dr. Christensen studied in Europe as a Guggenheim Memorial Fellow, and in 1950 he spent four months in Japan as a plant pathologist with the

Natural Resources Section, Department of the Army. As a result of his work in analyzing and evaluating disease problems in production and storage of Japanese food crops, he was made an honorary member of the Japanese Phytopathological Society. He is the author of many technical papers and he has served as Associate Editor of the journal *Phytopathology* and as President of the American Phytopathological Society.

**G. Arthur Cooper,** Curator of the Division of Invertebrate Paleontology and Paleobotany of the U.S. National Museum in Washington, D.C., has been awarded an honorary doctor of science degree by Colgate University.

**Morris B. Jacobs** has been appointed Director of the Laboratory of Air Pollution Control, Department of Air Pollution Control, City of New York. Dr. Jacobs has been a member of the staff of the City's Department of Health for the past 25 years, his last assignment having been that of Chief Organic Chemist of the Bureau of Laboratories.

**Herbert T. Kalmus,** President and General Manager of the Technicolor Motion Picture Corporation, Hollywood and New York, is a new member of the Board of Directors of the Stanford Research Institute, Stanford. Dr. Kalmus, a chemical engineer, is also President and General Manager of Technicolor, Inc. of New York and Chairman of the Board of Technicolor, Ltd. of London.

**John L. Kask,** a veteran of more than 25 years of activity in the field of fishery management and research, has resigned as Assistant Director of the Fish and Wildlife Service to accept the position of Chairman of the Fisheries Research Board of Canada, Ottawa.

**Thomas L. Kesler,** recently a geologist for U.S. Steel in the Alabama area, has joined Foote Mineral Company, Philadelphia, as geologist. His first assignment will be at Foote's Kings Mountain Mining Division.

The Medical Library Association has presented the Marcia C. Noyes Award for outstanding service in the field of medical librarianship to **Mary Louise Marshall,** Librarian of the Orleans Parish Medical Society Library, 1920 to date, and currently also Medical Librarian and Professor of Bibliography at the Tulane University School of Medicine. Miss Marshall has served the Medical Library Association in several capacities, this service being indicative of her loyalty to the Association and her foresight with respect to the needs of the profession.

**Lawrence Peters,** Associate Professor of Pharmacology at Western Reserve University, has been appointed Professor and Chairman of the Department of Pharmacology at the Tulane University School of Medicine. Dr. Peters succeeds **Ralph G. Smith,** who resigned in 1950 to accept an appointment as Medical Officer for the U.S. Food and Drug Administration.

**Geoffrey William Rake,** Coordinator of the Office of Scientific Affairs of E. R. Squibb and Sons Division, Mathieson Chemical Corporation, has been named a member of the Board of Directors of the American Institute for the Tropics, New York, and Chairman of its Committee on Medicine and Public Health. He will leave shortly for international scientific congresses in Istanbul and Rome.

**Julius B. Richmond,** Professor of Pediatrics at the University of Illinois and Superintendent of the Institute for Juvenile Research, has been appointed Chairman of the Department of Pediatrics at the State University of N.Y. College of Medicine. He will be the first full-time professor of pediatrics on the college staff. He succeeds **Tyree Wyatt,** who administered the Department on a part-time basis and who will continue his participation in the teaching program as Clinical Professor of Pediatrics. Dr. Richmond was for three years co-editor of *Yearbook of Pediatrics* and was also one of the authors of *An Introduction to Pediatrics.*

**Augustus B. Wadsworth,** formerly Director of the Division of Laboratories and Research of the New York State Department of Health, has been awarded the Hermann M. Biggs Medal for outstanding service to public health. The medal, established by the New York State Public Health Association, was presented to Dr. Wadsworth at the Association's annual meeting.

**Daniel Thomas Watts** of the University of Virginia Medical School has been appointed Professor and Head of the Department of Pharmacology in the West Virginia University School of Medicine.

**Kurt F. Wendt,** Professor of Mechanics and since 1948 Associate Director of the Wisconsin Engineering Experiment Station, has been named Dean of the University of Wisconsin College of Engineering. Dr. Wendt, who recently returned from a 20-day flying trip to India to conduct a technical education and research survey for the U.S. State Department, succeeds **Morton O. Withey** who retired July 1. **William R. Marshall, Jr.,** Associate Professor of Chemical Engineering, has been appointed Associate Dean of the College of Engineering and Associate Director of the Wisconsin Engineering Experiment Station.

**Stephen Wilson,** former Vice Dean of the School of Pharmacy at the University of Pittsburgh, has been appointed to succeed **Roland T. Lakey,** Dean of Wayne University's College of Pharmacy, who retired this June after 29 years at the University.

**Henry M. Woodburn** has been appointed Dean of the Graduate School at the University of Buffalo, effective July 1. Since 1945 Dr. Woodburn, an inorganic chemist, has been Chairman of the U.B. Chemistry Department.

## Education

**United Cerebral Palsy** has arranged five summer workshops designed to train teachers in the care and education of children with cerebral palsy. They will be held at the Universities of Arizona, Texas, and Nebraska, at San Francisco State College, and at Teachers College of Columbia University. The workshops were arranged by Maurice H. Fouracre, Head of the Department of Special Education at Teachers College, who is Chairman of the UCP Educational Advisory Board.

Catalogues of courses to be offered by the new College of Letters and Science of the **University of California** at Riverside are now available at the office of the registrar. Applications for admission to the four-year liberal arts college, which opens next February, must be filed by Jan. 15.

The **University of Vermont College of Medicine** was chosen as one of five medical schools in the United States to receive a $1000 scholarship award, which is given annually by the Mead Johnson Company, manufacturers of infant foods, to a graduating senior to enable him to take a year of general practice residency following his internship. The UVM recipient is Delbert D. Griffith.

## Grants and Fellowships

Dietmar Seyferth, a 24-year-old German-born graduate student at Harvard University, has been chosen to receive the first $2000 **Charles Lathrop Parsons Scholarship** of the American Chemical Society. Mr. Seyferth was selected by Charles Lathrop Parsons, former Chief Chemist of the United States Bureau of Mines and Secretary of the Society from 1907 to 1945, for whom the scholarship is named. Dr. Parsons, now 86, was chosen last December as winner of the Society's first **Charles Lathrop Parsons Award.** The award confers on the recipient the privilege of designating the next Parsons Scholar, who may study for one year at any institution he selects and in any field, provided it has some relationship or value to chemistry or chemical engineering. The award will be presented no more than once in every three years to a chemist or chemical engineer chosen solely for public service activities.

There were 13 anthropologists, 2 geographers, and 3 sociologists included in the group of 97 **Ford Foundation** Foreign Study and Research Fellowships awarded last month. The average age of the recipients was 29, and the awards were for periods of 1 to 2½ years for studies in Asia or the Near and Middle East. It is expected that a similar program, a third series of Foreign Study and Research Fellowships, will be announced next fall.

The **Fund for the Advancement of Education** has granted 290 fellowships to high school teachers in the U.S. and possessions, for the academic year 1953–54. The grants, aggregating approximately $1,500,000, are designed to enable the recipients to forgo regular teaching duties for a full year, and to pursue self-designed programs to deepen their liberal education, improve their teaching ability, and increase their effectiveness as members of their school systems and communities. Grants were made to 140 men and 150 women; 184 were in the social sciences, 48 in mathematics and the natural sciences, and 24 in the arts, business administration, physical education, and industrial arts.

The **George Washington University** has received from Eli Lilly and Co. two grants, totaling $10,250, for medical research in the value and effectiveness of certain vaccines and drugs used in combating infant and childhood diseases. John P. McGovern, Assistant Professor of Pediatrics, will direct a study of the immunity to whooping cough that is developed in infants by the use of new vaccines. Josephine Kety and Leroy Hoeck, medical officers in pediatrics, will share with Dr. McGovern the direction of a study of the value of erythromycin in treating infant and childhood diseases.

The **Lowell M. Palmer Fund,** established this year by Carleton H. Palmer in memory of his father, and administered by Cornell University Medical College, has announced the recipients of the first six awards under the Lowell M. Palmer Senior Fellowships in Medical Sciences. They are: $8000 to Joseph Dancis, New York University College of Medicine, whose field is Physiology of the Newborn; $6000 to Franklin W. Heggeness, University of Rochester, in the field of Physiology of the Gastrointestinal Tract; $8000 to Charles A. LeMaistre, Cornell University Medical College, in the field of Chronic Bacterial Infections; $6000 to William R. Milnor, Johns Hopkins University School of Medicine, in Cardiovascular Research; $8000 to David Karzon, University of Buffalo, in the field of Viral Infections; $8000 to Maurice S. Raben, Tufts College Medical School, in the field of Pituitary Hormones.

The **National Research Council of Canada** has granted 226 scholarships in science and engineering for 1953–54. Twenty-four special scholarships awarded for study abroad are to be held in the following universities: eight at London, four at Cambridge, three at Oxford, two each at Birmingham and North Carolina, and one each at the Massachusetts Institute of Technology, and the universities of Chicago, Delaware, Pennsylvania, and Wisconsin. Overseas postdoctoral fellowships have been granted for work at the following universities: three each at Oxford, Cambridge, and London, and one each at the National Institute for Medical Research, and the universities of Birmingham, Leiden, Louvain, and Uppsala.

Grants in aid of research amounting to more than $135,000 since April 14 have been announced by **Research Corporation,** a nonprofit foundation which has distributed approximately $7,500,000 in grants

since it was established by the late Frederick Gardner Cottrell in 1912. The Advisory Committee, whose recommendations were the basis for the current grants awarded, will meet again in September to consider further applications made during the summer. The grants, ranging from $400 to $8700, were made directly to U. S. colleges and universities, to provide support for specific research projects at early, critical stages of their development. Of the 64 grants announced, 45 were awarded under the Cottrell grants program, which aids science and teaching in the smaller colleges. The remaining 19 go to institutions under the general grants program, to support research of a pioneering nature. Among the projects to be supported by the grants are investigations on the interactions of cosmic rays, a new system of electrochemical analysis, and the potassium-argon method of dating geological specimens.

**UNESCO's Advisory Committee on Arid Zone Research,** at its fifth semi-annual meeting in Paris during the week of May 11, authorized grants totaling $7675 for five new research projects and proposed the publication of a comprehensive guide for the collection of information needed for the improvement of living conditions in semiarid and arid areas. The committee also chose Bangalore, India, for a symposium on wind and solar energy to be held in the autumn of 1954. The research grants are: $2835 to Jaswant College, Jodhpur, India, where Daya Krishna, Professor of Zoology, will study the habits of the vertebrate animals in relation to the local vegetation in the Desert of Rajputana, and their role in maintaining or spreading desert conditions; $2000 to the International Geographical Union for a study of the geographic potentiality of coastal deserts in Argentina, Australia, Mexico, Pakistan, Peru, and the Middle East; $1000 toward an international expedition to explore the Sahara and Sudan arid regions under the direction of Franz Kollmannsperger, zoologist of the University of Saarbrücken; $2000 to the Fuad I Desert Institute at Heliopolis, Egypt, for a symposium on desert research and development in Egypt and the Arab countries, to be held in Cairo in September; $840 to the Institute of Biology in Great Britain for the publication of the proceedings of the symposium held in London in 1952, on the Biology and Productivity of Hot and Cold Deserts.

## In the Laboratories

Three new appointments have been made at the **Los Alamos Scientific Laboratory** of the University of California. Nelson Jarmie, previously of the Radiation Laboratory of the University of California at Berkeley, will join the Experimental Physics Division; James M. Kister, who was pursuing graduate study at Harvard, will be in the Theoretical Physics Division; and Dean F. Mitchell, previously of the Guide Lamp Division of General Motors, will join the GMX Division at Los Alamos.

The **Mount Desert Island Biological Laboratory,** Salisbury Cove, Maine, has received a 3-year grant from Eli Lilly and Co. Each year $550 will be available for general support of the summer program, which features basic research in the fields of renal physiology and electrolytic balance, and of tissue culture.

Paul H. Scrutchfield and Walter J. Thome have joined the General Research Organization of **Olin Industries, Inc.** They are chemists in the film and cellulose section and are located in the East Alton plant, Company headquarters.

Creation of an Industrial Economics Department by **Southwest Research Institute** has been announced. Concerned largely with economics in the technical and production fields, the new department was established June 1 with the appointment of C. A. Harrell as chairman.

One of the first academic installations of a completely air-conditioned and temperature-controlled laboratory for research in the chemistry of refrigerators and refrigerating systems has been completed at the **University of Miami.** It will be under the direction of Walter O. Walker, Dean of the University's Division of Research and Industry.

## Meetings and Elections

The 19th Annual Meeting of the **American College of Chest Physicians,** held in New York City, May 28–31, surpassed all previous records for attendance. Approximately 1500 registered, including 1200 members and guest physicians, and 300 non-physician guests. The following officers were elected for the year 1953–1954: president, Alvis E. Greer, Houston, Tex.; president-elect, William A Hudson. Detroit, Mich.; 1st vice president, James H. Stygall, Indianapolis, Ind.; 2nd vice president, Herman J. Moersch, Rochester, Minn.; treasurer, Charles K. Petter, Waukegan, Ill.; assistant treasurer, Albert H. Andrews, Jr., Chicago, Ill.; chairman, board of regents, Donald R. McKay, Buffalo, N.Y.; historian, Carl C. Aven, Atlanta, Ga.

At the 78th Annual Meeting of the **American Neurological Association** the following officers were elected for the year 1953–54: president, Roland P. Mackay; president-elect, Percival Bailey; first vice president, William G. Lennox; second vice president, Russell Meyers; secretary-treasurer, H. Houston Merritt; assistant secretary, Charles Rupp.

**Cornell Aeronautical Laboratory, Inc.,** has selected officers for the fiscal year 1953–54. Reelected to head the Buffalo research laboratory were: president and chairman of the board, Theodore P. Wright; executive vice president and director of the laboratory, Clifford C. Furnas. The laboratory is a non-profit and self-sustaining affiliate of Cornell University, and operates as a corporate entity in Buffalo, New York.

The **Federation of American Scientists** has recently installed its officers for 1953–54. The new chairman is David L. Hill, Professor of Physics at Vanderbilt University and the vice-chairman is Edward U. Condon, Director of Research at the Corning Glass Works. Serving with them on the Executive Committe are Lewi Tonks of G.E.'s Knolls Atomic Power Laboratory (secretary), Hugh C. Wolfe, Head of the Physics Department at The Cooper Union School of Engineering (treasurer), Jules Halpern, Professor of Physics at the University of Pennsylvania, M. Stanley Livingston, Professor of Physics at M.I.T., and Clifford Grobstein of the National Institutes for Health, Bethesda, Md. The role played by the Federation in bringing the implications of the Astin dismissal to the attention of scientists and the public has resulted in a considerable increase in membership.

The U.S.A. National Committee of the **International Scientific Radio Union** (URSI) is sponsoring a Fall Technical Meeting jointly with the Canadian National Committee, URSI, and the IRE Professional Group on Antennas and Propagation. The meeting will be held at the National Research Council, and the Defence Research Board, Ottawa, Canada, October 5–8, 1953. This is the first meeting jointly sponsored by the U.S.A. and Canadian National Committees of URSI. Further information about the newly-formed Canadian Committee is available from its secretary, J. C. W. Scott, Defence Research Board, Ottawa, Canada.

The 1st Annual Meeting of the **Inter-Society Cytology Council** will be held in Philadelphia, November 19–20. All those interested in presenting papers pertaining to the cytologic method for the diagnosis of cancer and related subjects are urged to send titles, together with an informative abstract of not more than 300 words, to the Chairman of the Program Committee, Dr. A. E. Rakoff, Jefferson Medical College, 1025 Walnut St., Philadelphia 3, Pa. All titles and abstracts must be received before August 1.

## Miscellaneous

A non-profit foundation established for the purpose of assisting worthy projects in psychological education and research has been incorporated as a sister organization to the American Psychological Association. The new group is known as the **American Psychological Foundation.** It will be administered by a Board of Trustees made up of the seven past presidents of the A.P.A. They are: J. McV. Hunt, University of Illinois; R. R. Sears, Stanford University; J. P. Guilford, University of Southern California; E. R. Hilgard, Stanford University; D. G. Marquis, University of Michigan; C. R. Rogers, University of Chicago; and H. E. Garrett, Columbia University. The Foundation's principal offices will be in the new $250,000 building at 1333 16th St., N.W., Washington 6, D.C., recently purchased by the A.P.A. Since the Association publishes ten journals as well as a directory of its 11,000 psychologist members, it had a real need for its new headquarters.

The **American Museum-Hayden Planetarium,** New York City, will offer its streamlined courses in basic astronomy, navigation and meteorology especially designed for the layman, beginning in September. Each course includes a lecture and demonstration once a week presented informally by a member of the Planetarium staff, utilizing the unique projection facilities of the Planetarium. Interested prospective students are invited to attend the free introductory sessions offered at the start of each course.

No objection was raised to HR5016 in a House Interstate Committee hearing. The bill would strike "**aureomycin**" from the Federal Food & Drug Act and substitute the chemical term "chlortetracycline." Passage of the bill is required before Lederle can apply for registration of "Aureomycin" as a trade mark which in turn, is a requisite for curbing unauthorized use of the name in pharmaceutical manufacture abroad.

The proceedings of the 1952 **Biology Spring Conference** on "Some Aspects of Microbial Metabolism," held at Oak Ridge National Laboratory on April 10–11 of last year under the sponsorship of the ORNL Biology Division, have been published as Supplement I, Volume 41, March, 1953, to the *Journal of Cellular and Comparative Physiology.* A limited number of the 282-page volumes are available without cost to specialists in the field. Requests should be made in writing to the Biology Division, Oak Ridge National Laboratory, Post Office Box P, Oak Ridge, Tenn.

June 7th marked the 200th anniversay of the **British Museum.** Sir Hans Sloane, called "the father of natural history," died in 1753 and placed his valuable private museum at the disposal of his country. A special Act of Parliament later that year accepted the gift and created the nucleus of today's British Museum, which was first opened to the public in 1759.

An expanded editorial policy and a new editorial board have been announced for the journal **Human Biology.** A grant from the Wenner-Gren Foundation for Anthropological Research, which will insure publication of a full volume this year, will also enable the editors to widen the scope of interests represented in the journal. Gabriel Lasker of the Department of Anatomy of Wayne University is Editor, and associated with him will be a five-man editorial board embracing a range of interests from evolution to biostatistics. They are: Joseph Brožek, Laboratory of Physiological Hygiene, University of Minnesota; Bentley Glass, Department of Biology, The Johns Hopkins University; Donald Mainland, Department of Medical Statistics, New York University; James N. Spuhler, Institute of Human Biology, University of Michigan; and William L. Straus, Jr., Laboratory of Physical Anthropology, The Johns Hopkins University.

# *Technical Papers*

## Biophysical Methods for the Assay of the Life Span of Red Blood Cells[1]

Harold C. Box, Worthington G. Schenk, and Charles E. Wiles[2]

*Surgical Laboratory, Meyer Memorial Hospital, Buffalo, New York*

Radioactive iron, principally $Fe^{59}$, has been used frequently as a tracer in the study of red cell dynamics. This isotope which can be incorporated into the erythrocyte *in vivo* remains in the cell during its life span. However, upon the death of old cells, the iron is almost quantitatively reincorporated into new erythrocytes thus rendering uncertain the interpretation of many investigations. The rapid reutilization of iron has been demonstrated at this laboratory during the course of our investigations of the fate of transfused red cells. In an effort to avoid this difficulty we have been led to a consideration of other isotopes as erythrocyte tracers. The work of Shemin and Rittenberg (*1*) indicates that $N^{15}$ can be satisfactorily incorporated into the erythrocyte *in vivo*. An experimental study was undertaken to compare the relative reutilization of $Fe^{59}$ and $N^{15}$ after the destruction of the tagged erythrocytes.

The red cells of the dog are easily tagged by administering an intravenous dose of $Fe^{59}$ as a buffered solution of $FeCl_3$. The radioactivity in the red cells thus tagged is determined by first ashing a few cc of red cells and electroplating the iron out of solution following essentially the method described by Peacock *et al.* (*2*). The activity (in counts per minute) of the sample can then be measured with a conventional Geiger counter and the activity per cc of red cells calculated. In order to determine the total circulating activity, red cell mass measurements were made by the T-1824 dye technique.

The reutilization of iron was amply demonstrated in our laboratory when whole blood from a donor dog tagged with $Fe^{59}$ was transfused to an untagged dog. The donor's blood type did not match that of the recipient with the result that the recipient underwent a severe hemoclastic crisis following the transfusion. The destruction of the labeled cells and the subsequent reincorporation of the iron in newly formed cells is illustrated in Fig. 1 where the total circulating activity in the recipient is plotted against the day of the experiment. The days are counted from the day of the transfusion. The total activity measured in the recipient immediately after the transfusion is arbitrarily taken as 100%. The ordinate has been corrected for the natural decay of $Fe^{59}$ (half-life of 46.3 days) and for the effect of sampling losses. It is evident that 11 days after the transfusion nearly all the donor cells had been removed from circulation. Subsequently, however, approximately two-thirds of the iron in these cells was incorporated in new cells and restored to circulation. The work of Cruz *et al.* (*3*) further illustrates the reutilization of iron in the dog.

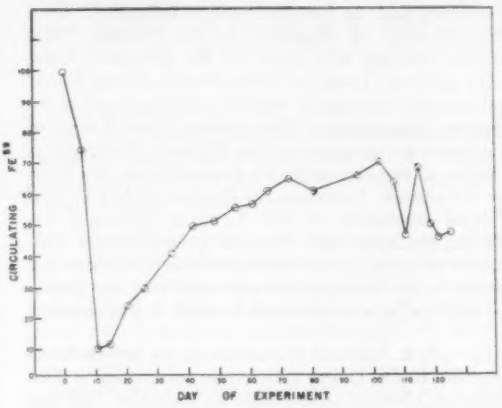


FIG. 1.

This characteristic makes the use of iron unsuited as a tracer for following transfused red cells over an extended period of time since one cannot determine what part of the activity is due to radioiron reincorporated in newly formed cells. The comparison of $Fe^{59}$ and $N^{15}$ in this regard was accomplished by double tagging of the erythrocytes of a healthy adult mongrel dog with $N^{15}$ and $Fe^{59}$. A week after the tagging with the radioiron, 19 g of $N^{15}$-tagged glycine prepared after the method of Schoenheimer and Ratner (*4*) were given orally in small doses over a 2-day period. The animal was offered only water during this time to insure maximum absorption of the glycine. Twenty-six days after this feeding (sufficient time to allow incorporation of both elements into newly formed red cells) a hemoclastic crisis was artificially produced. Four doses of 0.3 g of acetylphenylhydrazine were administered over a 4-day period by subcutaneous injection. Measurements of the red cell mass by the T-1824 dye method before and after the injections showed that the red cell mass had been decreased approximately 64%.

Periodic determinations of the red cell mass, $Fe^{59}$ radioactivity, and $N^{15}$ excess in the red cells were made. For this analysis 45 cc of whole blood were required. The red cell mass determination required 20 cc of blood, but the same aliquot could be used for the radioiron determination. The analysis of the remaining 25-cc aliquot for $N^{15}$ was somewhat more difficult

[1] This investigation was supported by a research grant from the Veterans Administration. The mass spectrometer used was made available to the University of Buffalo through a Grant-in-Aid from the American Cancer Society of the Committee on Growth of the National Research Council.

[2] We wish to thank Miss Sarah Camiolo for her competent handling of the chemistry associated with this research.

since this is a stable isotope. Hemin crystals were first obtained from the whole blood. By means of a micro-Kjeldahl process the nitrogen was extracted from the hemin in the form of an ammonium salt which was then treated in an evacuated system with hypobromide so as to release the nitrogen in gaseous form. The gas was compressed into sample bottles suitable for introduction into a mass spectrometer by means of which the ratio of $N^{14}N^{15}$ to $N^{14}N^{14}$ was determined. The probability of forming a $N^{15}N^{14}$ molecule is proportional to $2n_{14}n_{15}$ where $n_{15}$ and $n_{14}$ are the numbers of heavy and light nitrogen atoms in the sample. Likewise, the probability of forming a $N^{14}N^{14}$ molecule is proportional to $n_{14}n_{14}$. The ratio of $N^{14}N^{15}/N^{14}N^{14}$ will be therefore $2n_{15}/n_{14}$. It follows that the percentage of $N^{15}$ in the sample is

$$\% \, N^{15} = \frac{100 n_{15}}{n_{14} + n_{15}} = \frac{100}{2/r + 1}$$

where $r$ is the ratio measured on the mass spectrometer. The percentage of $N^{15}$ excess is obtained by subtracting 0.368 (the percentage of $N^{15}$ occurring naturally in matter) from this number. These procedures are essentially those described by Rittenberg *et al.* (*5*).

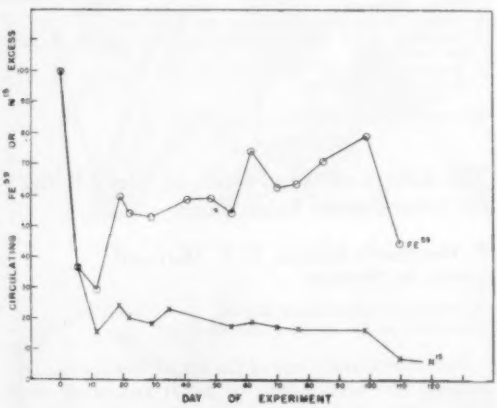


FIG. 2.

The results of the experiments are shown in Fig. 2. The circulating radioactivity as deduced from red cell mass determinations and radioactivity measurements was calculated at intervals of several days and is shown by the upper curve in the figure. The amount of activity just before the injection of the acetylphenylhydrazine was arbitrarily chosen as 100%. As usual, allowance has been made for sampling loss and the natural decay of the iron. The amount of circulating iron decreases sharply with the destruction of the dog's erythrocytes, but again there is remarkable reutilization as the dog regenerates its red cell mass.

In the case of the nitrogen it is the product of the percentage excess of $N^{15}$ and the red cell mass which is of interest. This we refer to as the circulating $N^{15}$ excess in Fig. 2 where it is plotted as a percentage of the initial value. Evidently the destruction of the red cells results in a permanent loss of $N^{15}$ since there is no measurable reutilization.

It is clear that by using an isotope of nitrogen rather than of iron to follow the fate of transfused red cells, the problems resulting from reutilization can be avoided. Moreover, since it is a stable element it is not subject to the restrictions of a long half-life radioactive tracer such as $C^{14}$—nor is it hampered by the uncertainties of the agglutination technique sometimes used to follow red cells which is a statistical method and depends upon the strength of the serums used. It must be noted, however, that extension of this technique to tracing of transfused cells in human beings, although feasible, will be difficult because of the high excess of $N^{15}$ required in the donor. Because of the inherent difficulties and uncertainties of other methods of following transfused red cells and in view of the results of this experiment, it is believed that $N^{15}$ can become an important tool in the nation's vital research program for improved blood preservation.

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## The Effect of Streptomycin on Oxygen Uptake and Viability of Resting Suspensions of *Escherichia coli*


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The inhibitory effect of streptomycin on the respiration of "resting" bacterial suspensions has been noted by several observers (*1–4*). Work in this laboratory has indicated that there is a discrepancy between the killing effect of streptomycin on bacterial cells and respiratory inhibition by this drug. The experiments reported in this preliminary note may be of interest from the standpoint of studying the effects of metabolic inhibitors on resting cell suspensions.

Simultaneous experiments, using the same suspension of *E. coli* (841),[1] were set up to compare the effects of streptomycin on oxygen uptake and viability. The results of such an experiment are presented in Fig. 1.

[1] The organisms were grown for 24 hr with aeration at 37° C in a synthetic medium prepared as follows: $(NH_4)_2SO_4$ 2.5 g, $KH_2PO_4$ 2.0 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, glucose 10.0 g (autoclaved separately), tap water 200 ml, distilled water to 1000 ml, pH adjusted so that after autoclaving pH was approximately 7. After harvesting, the cells were washed twice with sterile distilled water, suspended in sterile distilled water, and refrigerated at 4° C for 24 hr before use.

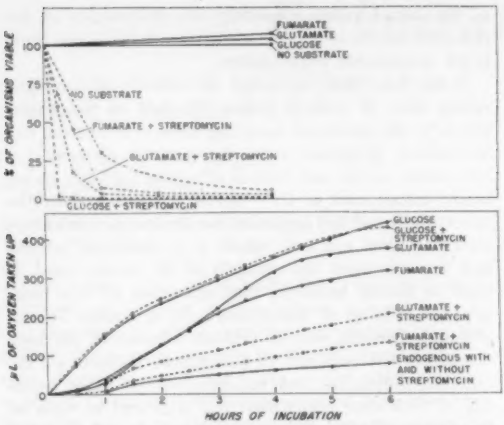


FIG. 1. Effect of streptomycin on respiration and viability of *E. coli*. The oxygen uptake studies are shown in the lower portion of the figure; conventional Warburg techniques were used. Fifty-milliliter Erlenmeyer flasks were used for the viability tests, shown in the upper part of the figure. The same suspension of cells was used in both instances and the ratios of cells to substrate and to streptomycin were kept the same, as follows: cells, 0.5 mg/ml (dry weight) containing approximately $2 \times 10^9$ viable cells; substrates, 5 μmoles; streptomycin, (dihydrosulfate) 100 μg/ml; phosphate buffer pH 7, 0.0036 *M*. The experiments were conducted at 37° C. with agitation to insure adequate oxygenation. Aliquots were removed at intervals for pour plate counting in heart infusion agar (Difco) with 1% glucose.

Streptomycin is noted to have no effect on endogenous respiration or on respiration in the presence of glucose, whereas there is a marked inhibition of oxygen uptake with fumarate and glutamate as substrates. Noticeable inhibitory effects become apparent after 1 to 2 hr of incubation. In the absence of streptomycin there is a lag period with glutamate and fumarate before the maximum rate of oxygen uptake occurs, whereas there is no lag period in respiration with glucose as substrate.

Killing of the cells by streptomycin is most rapid in the presence of glucose as substrate, with over 99% of the cells being killed in the first 15 min. Despite this fact, however, oxygen uptake with glucose as substrate continues in an unaffected fashion. The rates of killing are less rapid with glutamate and fumarate as substrates and least rapid in the absence of added substrate. The killed cells could not be revived by repeated washing in an attempt to remove the streptomycin. Similar experiments using streptomycin-resistant and streptomycin-dependent variants of this strain of *E. coli* showed no killing or respiratory inhibition by streptomycin.

These results indicate that streptomycin can kill susceptible coliform cells in the resting state in the absence as well as in the presence of inhibitory effects on respiration. The inhibition of oxygen uptake by streptomycin seems to bear no particular relation to the killing effect of this drug but rather to the substrate being metabolized. In view of the lag phase demonstrated in Fig. 1 in the oxygen uptake curves with fumarate and glutamate as substrates, respiratory inhibition with these two substances by streptomycin may simply represent an interference with adaptive enzyme formation, as earlier suggested by other workers (*5*).

It is not possible to say categorically that the cells have already been killed before plating because viability can be determined with certainty only by subculture. The streptomycin may merely be "fixed" in the bacterial cells during the period of exposure and "death" may occur following the initiation of metabolic activities concerned with growth when the cells are transferred to a suitable culture medium. Perhaps streptomycin renders the cells "sterile," that is, they are not dead but cannot reproduce themselves.

In any case it is fair to say that the fixing of streptomycin to the susceptible site in the cell, with subsequent death of the cell, occurs most rapidly in the presence of metabolizable substrates and least rapidly with endogenous metabolism. The rate of fixing of the drug to the cells appears to be directly related to the rate or degree of metabolism of the cells.

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## The Effects of Wind-Drift of Weed-Killer on Some Puerto Rican Trees


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For several years one of the junior writers has been noticing the toxic effects of 2-4-D and other weed-killers on susceptible species of cultivated plants such as Sea Island cotton, *Gossypium barbadense*, and papaya, *Carica papaya*. The damage to these plants is particularly noticeable when they grow near fields of sugarcane in Puerto Rico, where weed-killers are used to control weeds.

Last year at the Isabela Substation an entire papaya grove of about 600 plants was seriously affected by 2-4-D sprayed 2000 yd or more to the east. The fine spray of the weed-killer carried by the trade winds from the northeast was powerful enough to affect all the papaya plants.

Since January 1952, the writers have been making field observations and taking numerous photographs of the effects of weed-killers on our flora. Detailed observations will be published elsewhere in the near future. However, it is of interest to note that two common species of trees in Puerto Rico serve as an

index to indicate the susceptibility of plants to weed-killers, particularly 2-4-D.

On the south coast of Puerto Rico, the "Santa María" or "emajagüilla," *Thespesia populnea*, a malvaceous tree commonly grown along the roadsides, serves as an index of susceptibility in this area of Puerto Rico. The distorted leaves of this tree (Fig. 1) show symptoms of 2-4-D toxicity at all times of year, but most noticeably during the spring, when "primavera" cane as well as ratoon cane are being sprayed with weed-killers. Even more conspicuous is the injury to another common roadside tree which grows more generally in the northern part of the Island: the "almendro," *Terminalia catappa* (Fig. 2). In the Naguabo-Humacao area many mature trees, 30 to 50 ft in height, have been observed that were killed by the effects of 2-4-D used in adjacent cane fields (Fig. 3).

FIG. 1. Foliage of *Thespesia*, showing effect of 2-4-D toxicity.

FIG. 2. Terminal twig of "almendro," *Terminalia*, showing effect of weed-killer.

FIG. 3. Dead and dying roadside trees of "almendro," *Terminalia catappa*, on the Naguabo-Humacao road, Puerto Rico. (Photos courtesy of the Photographic Department, Agricultural Experiment Station, Río Piedras, Puerto Rico.)

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## Effect of Irradiation with Cobalt-60 on Trichina Larvae

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A previous study of the effect of 200 kv x-rays on trichina larvae (*1*) showed that a dose of 750,000 r kills trichina larvae *in vitro*, whereas a dose of approximately 5000 r inhibits maturation of larvae to adult forms, and a dose of approximately 3500 r produces sexual sterility of the adult forms that mature from irradiated larvae. The present study was undertaken to determine the radiation dose from a cobalt-60 source that would produce the same effects.

Alicata and Burr (*2*) found that exposure of trichinous meat to 12,000 r of cobalt irradiation produced sterility in 60–100% of the adult female worms recovered from the intestinal tract of experimental rats 6 days after the latter were fed the irradiated larvae.

Trichinous rat muscle and isolated trichina larvae obtained by the digestion of trichinous rat muscle

TABLE 1

NUMBER AND AVERAGE PERCENTAGES OF FEMALE ADULT TRICHINAE HAVING EMBRYOS, AMONG DESIGNATED NUMBER OF FEMALE ADULTS EXAMINED, AS RECOVERED FROM GUT OF RATS 6 DAYS AFTER FEEDING ANIMALS 5000 LARVAE EXPOSED TO $Co^{60}$ RADIATION

| Radiation dose applied to larvae fed to rats r | Source of larvae fed to rats | Number of gravid female adult trichinae among adult females examined | | | |
|---|---|---|---|---|---|
| | | Rat number in group | | | Average % |
| | | 1 | 2 | 3 | |
| 0 | | 50 of 50 | 50 of 50 | 50 of 50 | 100 |
| 12,800 | Irradiated trichinous muscle | 0 of 50 | 1 of 50 | | 1 |
| 15,360 | | 0 of 50 | 0 of 50 | | 0 |
| 0 | | 50 of 50 | 50 of 50 | 50 of 50 | 100 |
| 10,240 | Irradiated isolated trichina larvae | 8 of 25 | 5 of 25 | 7 of 25 | 26.7 |
| 10,240 | | 1 of 50 | 0 of 50 | 0 of 50 | |
| | | 0 of 50 | 1 of 50 | 6 of 50 | 2.7 |
| 12,800 | | 4 of 25 | 6 of 25 | | 20 |
| 15,360 | | 0 of 4 | 0 of 7 | | 0 |
| 15,360 | | 0 of 1 | 0 of 1 | 0 of 3 | 0 |
| | | 0 of 4 | | | |

were placed in lusteroid tubes for irradiation, as in our previous study (*1*). The filled lusteroid tubes were then placed inside a cylindrical cobalt-60 irradiation unit for exposure at a rate of 1280 r/min (*3*). The radiation source at the University of Michigan has been described in detail elsewhere (*4*).[1]

Measurements were made to determine the maturation-inhibiting dose and sexual-sterilizing dose for both isolated larvae and larvae in trichinous rat muscle. Measurements of the lethal dose were made only on the isolated larvae.

In determining the maturation-inhibiting and sterilizing levels of radiation, doses ranging from 5120 r to 20,480 r were applied. Larvae, irradiated in muscle, were subsequently isolated by artificial digestion of the muscle with 1% pepsin and 1% hydrochloric acid. The radiation effect was measured by reduction in the ability of the irradiated larvae to mature in 6 days in the intestinal tract, and by the absence of encysting trichina larvae in the muscle tissue of test rats 30 days after ingestion of trichina larvae. Each rat was tube-fed with 5000 larvae. For each radiation dose, whether applied to isolated trichinae or trichinous rat muscle, at least 6 test animals were used. Three of the 6 rats were sacrificed after 6 days and the contents of the small intestines examined for adult worms. The number of adult worms was calculated (*6*). When adult worms were present, 50 female worms were examined for the presence within them of embryos. The other 3 rats were sacrificed at the end of 30 days and examined for larvae in the muscles. When larvae were found, the number present in the entire carcass was calculated (*6*). In all, 160 rats were used to obtain the data on sexual sterilization and maturation inhibition.

[1] The kilocurie cobalt source at the University is located in the Waste Fission Products Laboratory, an activity devoted to research on use of radioactive fission by-products and supported by the U. S. Atomic Energy Commission and the Michigan Memorial-Phoenix Project (*5*).

The lethal dose for isolated larvae was determined from motility measurements on larvae irradiated at 269,000, 512,000, 768,000, and 1,024,000 r. At the end of each period of irradiation and at 2 hr and 24 hr following irradiation, 200 of the irradiated larvae were examined on a warm stage (43.3° C) for evidence of motility; in each case the percentage of motile forms was recorded. (Nonmotile [dead] larvae, when killed by irradiation, often appear tightly coiled under the microscope, so that the mere examination for coiled and uncoiled states does not determine viability. Death due to irradiation does not usually cause the larvae to uncurl as do heat and other killing methods.) In addition to microscopic examination, 5000 irradiated larvae were fed to 6 test rats, as in the experiments to determine the sterilizing and maturation-inhibiting doses.

The examination for embryos, within adult female worms recovered from the gut 6 days after irradiation (Table 1), shows that a small number of females may develop embryos after a dose of 12,800 r, but that no embryos develop after a dose of 15,000 r. The dose of radiation with $Co^{60}$ necessary to cause sterilization of trichina while they are still encysted in rat muscle

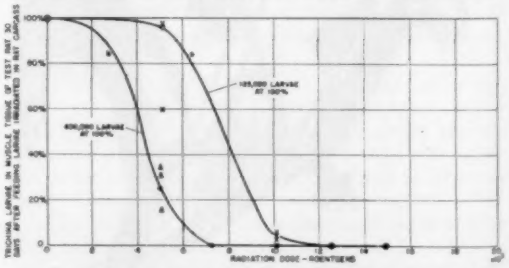


FIG. 1. Sterilization of isolated trichina larvae and of trichina larvae in rat carcass by $Co^{60}$ gamma irradiation. △ = isolated trichina larvae. × = larvae irradiated in rat carcass.

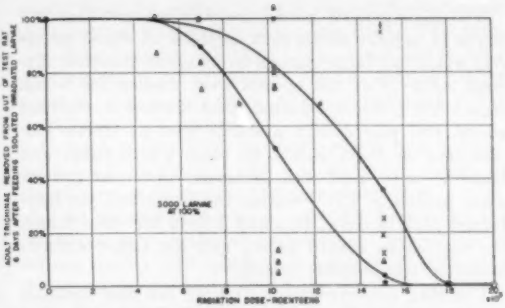


FIG. 2. Inhibition of maturation by $Co^{60}$ gamma irradiation of isolated trichina larvae and of trichina larvae in rat carcass. △ = isolated trichina larvae. × = larvae irradiated in rat carcass.

is indicated in Fig. 1. With a dose of 12,800 r over 99% of adult females were rendered sterile. Complete sterilization was reached by a radiation dose of 15,000 r. To achieve complete sterilization of isolated larvae irradiated *in vitro*, about 12,800 r were required (Fig. 1). These curves are based on the degree of infection found in muscle 30 days after feeding rats 5000 irradiated larvae.

By irradiation, it was possible to prevent larvae from maturing, even though the larvae showed normal motility in the warm-stage test. The amount of irradiation required to do this was somewhat higher than that for sterilization. When trichinous muscle was irradiated, 18,000 r reduced maturation to less than 1%. The variation in inhibition with dosage is shown in Fig. 2. The complete maturation-inhibiting dose for trichina larvae *in vitro* was 18,000 r (Fig. 2).

Radiation dosages required to kill were measured only on isolated larvae. Complete kill, as determined by the motility test, required 750,000 r. When the larvae were examined 2 hr after completion of irradition, the killing dose had dropped to 700,000 r, and when examined 20 hr after irradiation the killing dose was 400,000 r. From previous results, there is good reason to believe that the killing dose for irradiation of trichinous muscle would be substantially higher than the 700,000 r observed for irradiation *in vitro*.

Work is continuing on the irradiation of pork as a possible method of controlling trichinosis. Undesirable flavor changes occur in many foods when preserving doses of radiation (about 2,000,000 r) (*5*) are applied. However, one-hundredth of that dose is more than adequate to prevent maturation of encysted trichinae. Preliminary tests of pork irradiated with doses up to 38,400 r have shown negligible flavor change (*7*). A detailed report on this work will be published soon.

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## Some Applications and Limitations of Tetrazolium Chloride

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The use of 2,3,5-triphenyltetrazolium chloride (TTC) and its various derivatives in seed testing, in viability determinations, and in various other branches of research has become widely known. The reaction involving reduction of TTC seems to be essentially the same as that with methylene blue. However, TTC becomes colored when reduced, instead of colorless, and the oxidation reaction which reverses the color change reaction in methylene blue does not seem to occur or only occurs slowly under certain circumstances in TTC.

*Characteristics of the stain.* Young, rapidly growing tissues of such plants as corn and onion will show the red color in about 20 min when soaked in a 1% TTC solution. The carmine red color, which may alter to a cherry color in 24 hr, will also appear in solutions without the presence of living cells, e.g., when TTC is heated together with a reducing sugar to 50° C or subjected to strong light. With higher pH the light reaction will occur at lower light intensities for a given length of time. A higher pH also results in a more rapid reduction of TTC in living cells of a uniform kind (Table 1).

TTC-stained cells which appear red macroscopically may not show any red color under the microscope with an incandescent or daylight light source. It may, however, bring out the contour of mitochondria more clearly in such cells as those of corn coleoptiles. Other derivatives of TTC sometimes produce more striking effects.

The change in appearance of mitochondria was first demonstrated by H. Ziegler when the writer worked with him in B. Huber's laboratories in Munich. We have tried to reproduce his results in this country and also have observed the smaller mitochondria in corn coleoptile cells to be red after soaking for 2 hr in a 1% TTC solution. The color is variable depending on the depth of focus so that there may be some doubt as to whether the bodies are refracting light from in back of them or are giving off a color peculiar to the bodies themselves.

Chloroplasts may also become red in such cells as

[1] Aided by the School of Forestry, Duke University, Durham, N. C. We are indebted to P. J. Kramer for the use of his laboratory and to K. V. Thimann and C. S. Yokum for helpful suggestions.

TABLE 1

TIME OF APPEARANCE OF COLOR IN CORN ROOT TIPS AFTER IMMERSION IN 1% 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) SOLUTIONS OF VARIOUS pH (Temperature about 80° F)

| Min | pH | | | |
|---|---|---|---|---|
| | 3.0 | 5.4 | 7.8 | 8.4 |
| 5 | 0 | 0 | Pink | Red |
| 15 | 0 | Pink | Red | Red |
| 25 | Pink | Pink | Red | Red |

those of *Chlorella* and in most higher plant cells crystals of formazan later appear. As pointed out by Ziegler, the larger mitochondria of corn root tips, sometimes called statoliths, may also become reddish. If they do reduce TTC then they may serve as more than just starch synthesizing particles. It is not impossible to suppose that the electrical orientation of the tissue is dependent on their position and that this might affect the migration of auxin in roots or coleoptyles or in other tissues adjacent to statenchyma tissue and thus the bending of that tissue. It is known that cytoplasmic grouping to one end or another of a cell does effect the direction of the electric current (*1*) from certain kinds of tissue.

*Effect on Growth.* TTC may be applied in weak enough solutions and in short enough periods of time so that a stain will appear but the cells continue in a living state. Corn seed were allowed to germinate until the roots were about 5 cm long and then immersed in an 0.05% TTC solution for 5 min, removed, and washed in distilled water. The intact root together with the seed was then placed in a Petri dish on agar and observed for growth. In about 1 hr the color appeared and persisted for several days. It then slowly disappeared. Growth was somewhat reduced by this treatment in comparison to untreated controls. The stained roots responded to gravity by bending downward when the dishes were set on their sides.

TABLE 2

EFFECT ON 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) TEST AND ON NORMAL APPEARANCE OF *Pinus strobus* NEEDLES AFTER COOLING TO VARIOUS TEMPERATURES (°C)

| In TTC | Treatment | −7° | −10° | −13° |
|---|---|---|---|---|
| 5 min after thawing | TTC test positive | 100% | 40% | 2% |
| | Untreated leaves | All green | 50% light green | All light green |
| 2 hr after thawing | TTC test positive | 100% | 5% | 2% |
| | Untreated leaves | All green | 70% light green | All light green |

* Percentage of needles showing positive test 24 hr after placing in TTC. Determinations made in July.

The same general procedure may be used with embryos of conifer seeds, such as those of *Pinus palustris* which are large enough to be easily handled. Excised embryos of this species were washed for 3 min in a 0.5% Chlorox solution, then washed in distilled water, and placed with a sterile wire on sterile glucose-agar in Petri dishes. In cases where fungi and bacteria were kept out, embryos continued growth when soaked in TTC solution for 1 min and yet later turned pink in color. In about 3 days the color faded, the cotyledons turned green, and the entire embryo increased considerably in size.

*Viability determinations.* One of the best methods of determining the viability of conifer leaves after various drying and freezing treatments is with TTC. In needles that have been dried down over a 20-day period to about 40% of their moisture content based on dry weight, the TTC test showed a slow decline in intensity, also a decline in the total number of leaves showing a positive test. The rate of $CO_2$ evolution determined with the University of Munich infrared gas analyzer showed a decline at a slower rate than the TTC test, and with more rapid drying showed a slight rise before dropping to zero (*2*).

TABLE 3

TIME OF APPEARANCE OF COLOR IN EXCISED EMBRYOS OF *Pinus strobus* IN 1% 2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE (TTC) SOLUTIONS OF VARIOUS pH (Temperature 80° N)

| Seed treatment | Time since immersion in TTC | pH | | | |
|---|---|---|---|---|---|
| | | 3.3 | 4.2 | 7.3 | 8.3 |
| Dry stored seed | ½ hr | 0 | 0 | 0 | 0 |
| | 1 " | 0 | 0 | 0 | 0 |
| | 20 " | Pink | Pink-red | Pink-red | Pink-red |
| | TTC test* | 40% | 50% | 50% | 50% |
| Seed soaked in $H_2O$ for 11 days | ½ hr | 0 | Pink | Pink | Pink |
| | 1 " | Pink | Pink | Pink | Pink |
| | 20 " | Red | Red | Red | Red |
| | TTC test* | 100% | 100% | 80% | 75% |

* Percentage of 10 excised embryos showing a complete pink or red color in 20 hr. Variations of TTC % tests with pH are not statistically significant.

In determining the viability of frozen needles, the TTC test is less accurate. It is best to allow the material to stand for about 2 hr at room temperature before testing. The rate of $CO_2$ evolution may, like the TTC test, be about normal just after thawing of frozen damaged tissue, but it then declines to about one-half the normal rate in a half hour and in an hour nearly to zero. These rates were determined with the Duke University infrared gas analyzer. The TTC test showed the same general pattern of decline after thawing, but the TTC determinations took place over a 24-hr period so that results are more difficult to

assess. However, differences did appear depending on the length of time before immersing in the TTC solution after thawing (Table 2). The more badly damaged tissue (−13° C) can be seen to show a nearly negative test sooner than the less damaged tissue (−10°).

In seed testing TTC has been widely and successfully used. Evaluating the intensity and extent of staining so as to predict the germinability of a sampled lot of seed is the main difficulty. Sometimes the test seems to overrate the germinability percentage in certain conifer seed which have a period of dormancy that must be overcome for relatively rapid germination. This discrepancy may be partly the fault of the germination tests which are carried out with samples from the same lot of seed (*3*). We obtained the best predictions by making the TTC test on a sample of seed as they arrived from the seed house before stratification, and comparing these with percentages of germinating seed after stratification in moist cloth at 40° F for 2 months. Evidently there is not only a decrease in time of reacitvity of the seed to TTC during stratification, but there is an increase in the total percentage of seed showing a positive TTC test (Table 3).


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## The Influence of a Plasma Factor on *in vitro* Leucocyte Migration[1]


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While the intrinsic mobility of leucocytes is a well-known phenomenon, the influence of the plasma on this mobility has not yet been made clear. Using an *in vitro* method we have been able to show that the rate of migration of leucocytes is strongly influenced by a factor in the plasma.

In this technique, heparinized venous blood is first divided into cells and plasma. The cells are then washed three times by a sequence of suspension and centrifugation in Hanks' solution (*1*), and finally resuspended in autologous or homologous plasma. This suspension is then drawn into thin-walled capillary tubes of the type commonly used for melting point determinations. Each tube is sealed and then centrifuged to subdivide the system into three components; namely, a basal layer of packed red cells, an intermediate buffy coat of leucocytes, and an uppermost layer of plasma (Fig. 1). The initial concentration of heparin is adjusted so that the plasma clots after centrifugation, thus providing a matrix into which the leucocytes can migrate. Tubes are incubated in an upright position for 16 hr at 37° C, and the distance which the leucocytes have migrated into the plasma is then measured with an ocular micrometer. This measurement is therefore an indication of the distance which the fastest-moving leucocytes have traveled from the buffy coat. For each combination of cells and plasma, ten tubes were measured, and an average

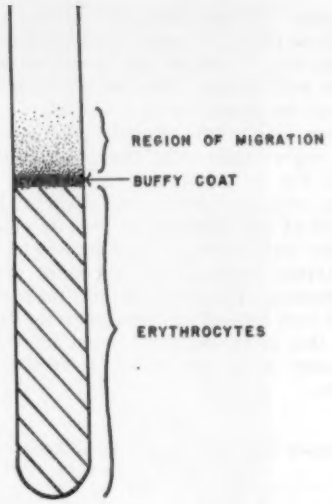


FIG. 1. Diagrammatic representation of the capillary tube method of measuring leucocyte migration.

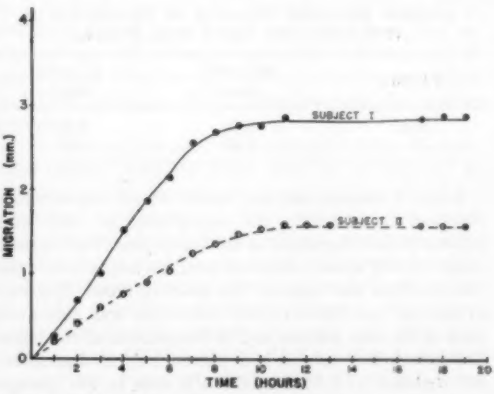


FIG. 2. Rate of leucocyte migration in two subjects with different migration distances. The difference in migration distance is the result of a difference in the rate of migration during the early hours of the experiment.


[1] Work done under grants from the United States Public Health Service, the Nell Lucas Forsythe Fund of the Harvard Medical School, and the Fund for Research in Bacteriology, Peter Bent Brigham Hospital.

[2] The authors wish to express their appreciation to Carroll M. Williams and Louis K. Diamond for their advice and criticism during the preparation of this paper.

value taken. The migrations are not free movements into a liquid phase. If a clot is present, the cells move along the fibrin micellae. But if no clot is present, the cells will migrate along the walls of the capillaries, and the results are very similar.

For the experiments, it was most convenient to make a single measurement after 16 hr. However, as shown in Fig. 2, rate studies indicate that when the migration distance between two subjects varies, this is a result of the difference in the rate of migration during the early hours of the experiment.

The normal subjects used for these experiments were laboratory personnel. At the time of the experiment each subject was asymptomatic. It was discovered that symptoms from slight infections were accompanied by a marked effect on the leucocyte migration.

TABLE 1

AVERAGE MIGRATION DISTANCE OF LEUCOCYTES FROM NORMAL SUBJECTS

| Subject | Sex | Blood type | No. expt | Mean (mm) | Standard deviation (mm) |
|---|---|---|---|---|---|
| KE | F | A, Rh + | 5 | 0.47 | 0.05 |
| JH | F | A, Rh + | 6 | 0.56 | 0.04 |
| MK | M | A, Rh + | 18 | 0.71 | 0.10 |
| JL | M | A, Rh + | 13 | 2.16 | 0.38 |
| EAS | F | O, Rh + | 5 | 2.47 | 0.61 |
| CBF | M | O, Rh + | 5 | 2.64 | 0.26 |

Table 1 records the data on experiments designed to evaluate the technique. The cells of normal persons were here suspended in the individual's own plasma. Though the cell migration of a given individual was relatively constant on different days, it is clear that a great deal of variation exists between individuals.

TABLE 2

AVERAGE MIGRATION DISTANCE OF LEUCOCYTES IN AUTOLOGOUS AND HOMOLOGOUS PLASMA

| Plasma | MK cells (mm) | JL cells (mm) |
|---|---|---|
| MK | 0.54 | 0.78 |
| JL | 1.63 | 1.93 |

Table 2 summarizes the results of six experiments designed to ascertain the importance of cells and plasma in the migration of the leucocytes. Two normal males having widely different average migrations were chosen. Both had type A, Rh positive blood. The migration of the cells of each individual was measured both in his own plasma and in the plasma of the other individual. MK cells in MK plasma migrated an average distance of 0.54 mm, and JL cells in JL plasma migrated an average distance of 1.93 mm. However, when MK cells were tested in JL plasma, their migration increased to 1.63 mm, and when JL cells were tested in MK plasma, their migration decreased to 0.78 mm. While there is still a difference in the migratory ability of the cells of the two subjects, it is evident that the plasma dictates to a large degree the rate of cell migration.

Similar experiments were repeated on types A, O, and B individuals. The results were consistent with the findings on the two initial subjects. In another series of experiments, the blood groups were mixed. Under such circumstances, if migration was not prevented by agglutination of the erythrocytes during the preparation of capillary tubes, the results were also consistent with those shown in Table 2.

The substance or substances contained in the plasma which condition the migration of leucocytes are heat-labile and nondialyzable. At present we are attempting to characterize the substances further by fractionation of the plasma proteins according to the small volume cold alcohol technique (*2*). Preliminary data indicate that when Fraction 3 of a slow plasma is added to a fast plasma, the migration of leucocytes in that plasma is markedly suppressed. And, when Fraction 2 of a fast plasma is added to a slow plasma, the migration of leucocytes in that plasma is increased.


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## Hepatic Tumors Due to Prolonged Ethionine Feeding[1]


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Ethionine (α-amino-γ-ethylthiobutyric acid) is an analogue of methionine and one of its biologic antagonists (*1, 2*). It produces, within a few days, fatty metamorphosis of the liver in female but not in male rats (*3, 4*). The lesion was considered the result of interference with amino acid or protein metabolism (*5*). Feeding synthetic diets containing 0.5% ethionine to male and female rats for 4 weeks produces a reduction of the fat content and severe liver cell damage (preceded by diffuse fatty metamorphosis and central necrosis). The liver shows diffuse infiltration by inflammatory cells as well as intralobular fibrosis and bile duct proliferation (*6*). Large liver cells with huge, sometimes multiple, nuclei and very large eosinophilic nucleoli are noted as well as irregularity in the appearance of the bile ducts (*7*). These pictures, interpreted as irregular regeneration, raised the question whether tumor formation may eventually result


[1] Supported by a grant from the Jerome D. Solomon Memorial Research Foundation.

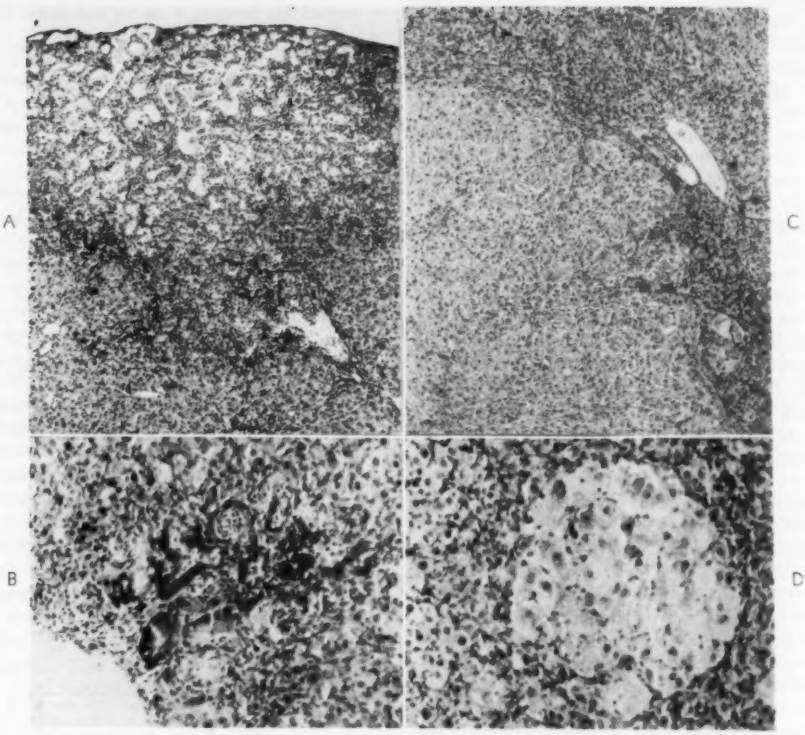


FIG. 1.

Photomicrographs of rat liver. *Upper left,* 0.2% ethinoine diet for 104 days. Subcapsular nodule consisting of proliferated bile ducts with dilated lumen containing mucinous material. The irregularly demarcated nodule connects with a portal triad (50 ×). *Lower left,* 0.5% ethionine diet for 51 days. Circumscribed heightening of bile duct epithelium with piling up of the cells and hyperchromasia of their cytoplasm and nuclei. Between the cells and in the lumen segmented leukocytes are found (112 ×). *Upper right,* 0.5% ethionine diet for 51 days. Fairly sharply demarcated nodule consisting of liver cells varying in character from the surrounding tissue in which the liver cells are irregularly arranged and dense inflammatory infiltration is noted (40 ×). *Lower right,* 0.5% ethionine diet for 51 days. Small nodule consisting of large liver cells forming several cell thick plates and revealing large nuclei and nucleoli and loose eosinophil cytoplasm (250 ×).

from this diet. Therefore, the attempt was made to keep rats (females) on these diets for longer periods.

The survival time varies markedly in different groups of rats on the 0.5% ethionine diet although most die within 5 weeks. Some groups, however, lived more than 50 days. Other groups of rats which became sick after 4 weeks were placed on a stock diet for 10 days, to be returned subsequently to the 0.5% ethionine diet. Finally, rats were kept for prolonged periods on diets containing 0.2% ethionine. All ethionine diets consisted of 16% casein, 75% sucrose, 5% corn oil with the addition of essential salts and vitamins; the riboflavin content was relatively low, being 0.42 mg/100 g. The diets had an approximate methionine content of 0.4%. Control rats on the synthetic diet without ethionine showed no changes.

Nodules consisting of either liver cells (hepatomas) or bile ducts (cholangiofibrosis or cholangiomas) were seen in 8 of 12 rats on the 0.5% ethionine diet for 51 days, in 1 rat on the 0.5% ethionine diet for 77 days, in 2 of 4 rats on the 0.2% ethionine diet from 87 to 104 days, and in 2 of 6 rats which had been kept on alternating 0.5% ethionine and stock diets between 60 and 67 days. Such lesions were not found in rats which had been on 0.2 or 0.5% ethionine diets for shorter periods.

The cholangiofibrotic or cholangiomatous nodules were usually multiple. They were up to 1½ mm in diameter and connected with portal triads (Fig. 1A). Some were subcapsular. Their epithelial cells were high cuboidal, densely arranged, and sometimes piled up. The basal nuclei were dark. The intensely basophilic cytoplasm occasionally contained vacuoles giving PAS (periodic acid-Schiff) reaction. The ductal lumen was dilated, occasionally cystic, and filled with a dense material also giving PAS reaction indicating a mucinous character. Segmented leukocytes were often found between the cells and in the ductal lumen. The basement membranes were thickened and dense collagenous and reticulum fibers surrounded the ducts

within the nodules. The demarcation of these nodules from the surrounding liver tissue was not sharp. Early changes appeared near the portal triads. They were alterations of the proliferating smallest bile ducts, characterized by heightening of the bile duct epithelium with hyperchromasia, piling up of the cells, and PAS reaction (not given otherwise by proliferating bile ducts) (Fig. 1B). This change occasionally involved only one segment of the wall of the bile duct.

The hepatomas, also usually multiple, consisted of nodules up to 2 mm in diameter which, on the greatest part of their circumference, were sharply demarcated from the surrounding liver tissue (Fig. 1C). The nuclei of the cells were, as a rule, large, vesicular, and showed huge nucleoli. The abundant cytoplasm was loose and eosinophilic. The cells were arranged in several cell thick plates with few capillaries and Kupffer cells between them (Fig. 1D). The nodules contrasted sharply to the liver tissue outside in which the inflammatory and fibrotic changes were marked and the liver cells had a denser cytoplasm and an irregular arrangement. The early stages of these nodules were small groups of liver cells (5 to 8 in a section) which represented focal multiplication of the liver cells so that the plates became several cells thick. The liver cells in these small groups resembled each other but varied from the surrounding tissue.

Hepatomatous and cholangiomatous nodules were found within the same liver. The pancreas of the animals showed severe fibrosis, atrophy of the acini, and vacuolization of the islet cells. These lesions represented a further advance of lesions previously described (*6, 8, 9*).

It appears, thus, that feeding diets containing an amino acid antagonist produces, in relatively short time and rather consistently, small multiple hepatic tumors composed of either liver cells or bile ducts varying in character from those in the surrounding tissue. The precursor stages of the nodule formation can be prevented or corrected by methionine administration. Although abnormal growth and anaplasia are seen histologically, it cannot be determined that the tumors represent malignant growths. No agreement is found in the literature as to whether the lesion called cholangiofibrosis or cholangioma represents neoplasia at all; this is reflected in the variety of names chosen for this lesion which is characteristically produced by carcinogenic dyes. It also cannot be ascertained whether the tumor formation is caused by ethionine directly or results from the excessive regeneration due to persisting injury to liver cells and bile ducts associated with diffuse fibrosis.

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## Compact Flowmeters for Use in the Unanesthetized Animal, an Electronic Version of Chauveau's Hemodrometer[1]


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Certain characteristics are desirable in a flowmeter for use in animal experimentation. These include (1) high sensitivity, (2) low resistance, (3) a regular and reproducible calibration, preferably linear, and (4) ease of use. Attempts to achieve these have extended over more than a century. Probably the first successful instrument was Chauveau's hemodrometer (*1*). The essential feature was hit upon while watching needles thrust into arteries. His first device consisted of a straight cannula with a rubber membrane as part of the wall. A lever extended from the center of the stream through this membrane, and movements of the lever were read against a calibrated scale. On a later

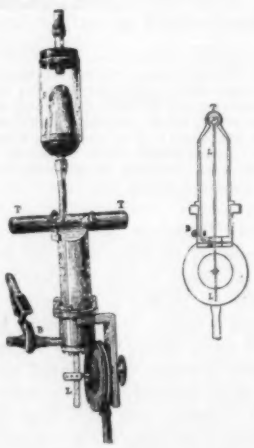

Fig. 1. Chauveau's hemodrometer as diagrammed by Luciani (*3*). The figure on the left shows the entire instrument, the cross section on the right is of the inverted, lower portion of the instrument. S, a sphygmoscope used to record the blood pressure; TT, a tube used to cannulate; L, a lever suspended by a rubber membrane at m. The lower end of the lever within the stream carries a paddlelike obstruction, while the outer end moves a tambour connected by tubing to a recording tambour.

[1] Aided by grants from the State of Washington Research Fund for Biology and Medicine and the National Heart Institute, USPHS.

modification (*2*), the hemodromograph (Fig. 1), the lever was connected to a recording tambour. With this it was first noted that blood was squeezed backward in the coronary arteries by ventricular contraction. The two cannula flowmeters to be described here prove to be in principle electronic versions of Chauveau's instrument of nearly a hundred years ago.

The first type of flowmeter consists of a T-shaped stainless steel tube. The stem or side branch of the T makes a friction fit with the body of an RCA 5734 vacuum tube (mechanoelectronic transducer) and the cross piece is used to cannulate and carries the flow to be measured (Fig. 2). An obstruction to flow lies in this crosspiece and is fastened only to the moving anode of the vacuum tube. Pressures exerted on the obstruction by the stream will cause movement of the sensing element and alter the output of the vacuum tube. A power supply, plate resistor, and meter or oscillograph are the only additional equipment necessary.

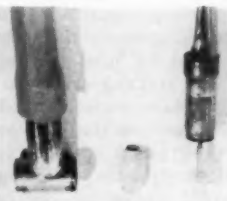

FIG. 2. A flowmeter employing the RCA 5734 tube. On the right the tube with a streamlined obstruction, in the center a flat paddle, and on the left an assembled flowmeter in stainless steel T-cannula. Diameter of the tube at its widest point is 0.375 in.

Two types of obstruction to flow have been used. One consists of a paddle of stainless steel which is placed across the stream to give "form" drag. The second is a streamlined rod or tube of plastic which offers "skin" or viscous drag. The paddle flowmeters may be made extremely sensitive by increasing the paddle cross section, but the calibration is that of a "$V^2$" device, that is, the deflection of the paddle is proportional to the square of the velocity. This characteristic results in severe distortion of wave forms and requires replotting of records where pulsatile flow is recorded. Viscous drag flowmeters are less sensitive, but their calibration approaches linearity (deflection = $kv^{1.2}$), and they offer less resistance to flow. The 5734 tube has ample sensitivity for use in a viscous drag flowmeter.

The mechanoelectronic transducers have been found to vary somewhat in characteristics. At very low flows they will change output due to cooling. For this reason plastic cannulas were considered less useful. It is possible that in animal use plastic will be more satisfactory than metal. In calibration the whole assembly was immersed in fluid, which minimized cooling effects. The vagaries of the vacuum tube are more evident with less sensitive flowmeters. Typical calibration curves are shown (Fig. 3).

A second group of flowmeters has been constructed

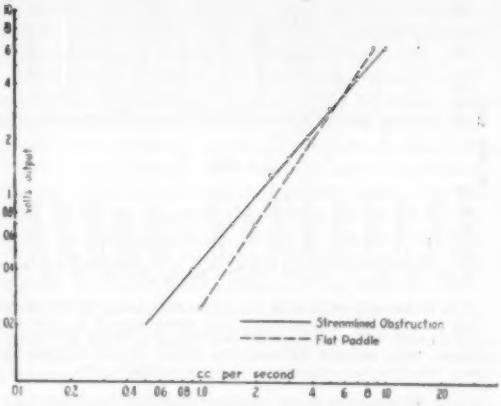


FIG. 3. Calibration curves for two flowmeters employing the 5734 tube with flat and streamlined obstructions.

in which the obstruction was of ferromagnetic material, and a plastic cannula wall incorporated windings whose inductance was altered by the movement of the obstruction. Some of these had both primary and secondary windings so that the whole constituted a differential transformer. With this design, the obstruction may consist of a flexible paddle, or a spring-suspended disk or cylinder. The windings may take several different shapes and the illustration presented (Fig. 4) is schematic for many that may be constructed. In these flowmeters, sensitivity is determined by the number of turns of wire, the magnetic permeability and movement of the element and its distance from the windings. A ferromagnetic frame for the windings will increase the sensitivity by increasing the amount of flux passing through the obstruction. Despite the use of all possible measures to achieve sensitivity, these flowmeters have never been sensitive enough to employ a streamlined obstruction with flow velocities such as are found in the dog's abdominal aorta. Although characterized by lower sensitivity than flowmeters using the 5734 tube, differential trans-

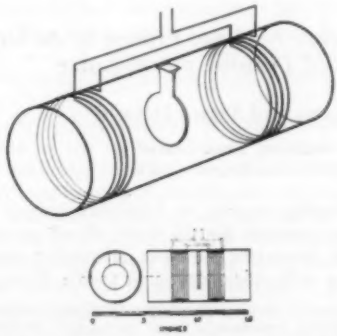

FIG. 4. Schematic diagram of a flowmeter in which a moving ferromagnetic obstruction alters the inductance of windings around a tube.

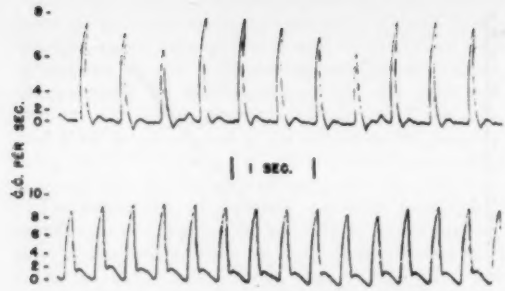


FIG. 5. Records of flow in the abdominal aorta in two dogs after recovery from anesthesia. The upper record is from an older animal which on autopsy was found to have arteriolar disease. This animal delivers his entire stroke volume in about one-fifth the time required for a cardiac cycle.

former flowmeters have greater stability and are not affected by cooling at low flows. Since they are incorporated in a straight tube, they may be easier to insert in certain vessels.

Differential transformer flowmeters require a stable source of alternating current for the primary. The output of the secondary is amplified and rectified by conventional techniques. Where only two windings are employed, these may be connected as adjacent legs of an inductance bridge.

The flowmeters illustrated have been constructed for the larger arterial vessels of the dog, but components may be varied in size for other vessels. They have been successfully employed in the abdominal aorta of the dog under anesthesia and in two animals have been placed in the abdominal aorta and have functioned for three and five days, respectively, after operation (Fig. 5).


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## Ribonucleic Acid and Protein in the Growing Oocytes of *Triturus pyrrhogaster*


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The growing oocytes of Amphibia present a very convenient material for the study of cell growth, and their yolk formation has been frequently studied in connection with ribonucleic acid (*1–5*). However, before we can discuss the chemical mechanism of yolk formation it seems necessary to accumulate exact data by different approaches. In the present paper the first sets of our experiments along this line will be reported, in which quantitative estimation of RNA and protein was performed on growing oocytes of *Triturus pyrrhogaster*, and the data obtained were compared with the histochemical picture of the same subject. Throughout the experiments rapidly growing winter oocytes before maturation were used.

*Histochemical.* As the fixative absolute alcohol, Zenker's fluid or 10% formalin was used. Sections were stained with methyl green-pyronin or toluidin blue. In the control series sections treated with ribonuclease or hot trichloroacetic acid were stained. The alcohol-fixed material gave the most beautiful staining with these dyes.

In young oocytes smaller than 0.05 mm$^3$, the cytoplasm was homogeneously and deeply stained, whereas the nuclear sap showed a granular staining. Many small nucleoli,[2] showing intensive basophilia, were attached to the internal surface of the nuclear membrane. In oocytes of about 0.7 mm$^3$ the germinal vesicle showed hardly any staining except in the nucleoli. The strongly basophilic nucleoli, which were somewhat larger in size and contained one or more refractory vacuoles, were found adjacent to the nuclear membrane. A relatively intense staining of the cytoplasm was observed around the germinal vesicle. In oocytes of about 2 mm$^3$ a very faint cytoplasmic staining was found on the external surface of the germinal vesicle. Most of the nucleoli appeared less basophilic, showing the above mentioned vacuoles and an irregular contour. In oocytes of 3 mm$^3$ the cytoplasm was fully laden with yolk granules, and they had almost completely lost their basophilia. The faintly stained nucleoli were found in the center of the germinal vesicle, whereas none were found in the periphery. This condition of both cytoplasm and germinal vesicle was observed also in later stages of the growth period. With respect to the behavior of the nucleoli, all fixatives gave approximately the same results. Our results are in good accord with the observations of Duryee (*7*) on the living oocytes of the same species.

The following facts made it probable that the basophilia of above-mentioned structures was due to RNA: (a) the stainability with pyronin and toluidin blue was completely lost by treating sections with crystalline ribonuclease prepared after the method of McDonald (*8*) or by extracting them with 5% trichloroacetic acid at 90° C for 15 min; (b) they were always Feulgen-negative. We have been careful not to accept such histochemical evidence as absolutely conclusive.

The results obtained on the prepared sections were essentially in agreement with those of Brachet (*1–4*) and Wittek (*5*). As repeatedly emphasized by Brachet,


[1] The authors wish to thank Tuneo Yamada under whose guidance these experiments were performed. Further, they express their appreciation to Barry Commoner, Eugene Roberts, and Florence Moog for critical reading of the manuscript. The investigation was aided by the Science Research Expenditure of the Department of Education.


[2] Some doubt has been expressed about identifying these structures as nucleoli (*6*). Without going into a discussion of this point, we provisionally adopt here the usual terminology.

these pictures give us an impression that the cytoplasmic RNA of the growing oocyte was rapidly decreasing during oogenesis. Similar results were also reported for oogenesis of other forms (*9–12*), and have been discussed in relation to the yolk-protein synthesis (*5*). However, the volume of the oocyte at the last growth stage under our examination was about 100 times as large as that of the initial stage. Therefore the decrease in concentration, judged by the stainability, does not necessarily mean a decrease in the absolute amount of RNA of an oocyte during the growth period.

*Biochemical.* The ovaries were removed from one to three females and immediately fixed with ice-chilled absolute alcohol. The oocytes were torn off from the ovarian epithelium, divided according to their diameter into groups of 15 to 60, and homogenized in ice-chilled absolute alcohol according to the directions of Ogur and Rosen (*13*).[3] Fractionation and photometric

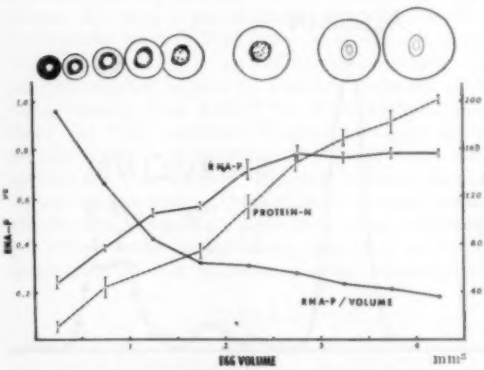


FIG. 1. RNA-P/oocyte, RNA-P/volume, and protein-N/oocyte compared with diagrams of basophilia during the growth of oocyte of *Triturus pyrrhogaster*. Each plotted value of RNA-P is the mean of 7 to 14 separate determinations. The value for protein-N is based on two to four separate determinations. Standard error is indicated for each value of RNA-P/oocyte and protein-N/oocyte curves.

estimation of RNA were made according to Schneider (*14*), and the amount of RNA thus obtained was expressed in terms of μg RNA-P/mm³ and of μg RNA-P/oocyte. Data are shown in Fig. 1, which is accompanied by histochemical pictures of each stage examined. As we have no exact knowledge about the temporal sequence of stages of growth of oocytes, obtained values were plotted against the size of oocytes. As will be seen from Fig. 1 the amount of RNA/cell progressively increased with its growth in the early phase of the period studied. However, beyond a certain point, the value remained almost constant in spite of increase in volume. On the other hand the concentration curve (RNA/mm³) fell sharply during the earlier phase and then declined gradually.

The general trend of the basophilia found in the sections of the growing oocytes seems to reflect the concentration curve of RNA-P. But closer comparison of this curve with the histochemical pictures suggests that it is very dangerous to predict any quantitative relation from the histochemical pictures alone. On the other hand, the diagram shows that the phase of increase in absolute amount of RNA coincides with the stages in which the strongly stainable nucleoli are applied to the internal surface of the nuclear membrane, whereas no increase in the absolute amount of RNA occurs in the later stages where the less stainable nucleoli are found only in the central part of the nucleus.

Brachet (*2*) reported that the young oocyte has a higher RNA content compared with the grown oocyte, calculating its value in terms of concentration per unit weight. On the other hand Villee and Duryee (*15*) found 2.05 times as much RNA-P in the larger oocyte as in the smaller one.[4] Our results also show that despite the apparent decrease of its concentration due to the increase in the volume of the cell, the absolute amount of RNA per oocyte does increase in the earlier phase of the growth period and does not decrease throughout the entire period.

The above estimations were carried out on the oocytes with the follicular epithelium. Although our histochemical study showed no detectable amount of RNA in the follicular epithelium, it seemed necessary to know whether the presence of the latter does not interfere with the estimation of the amount of RNA in the oocyte. To check this point the follicular epithelium was operatively removed in 1 *M* NaCl containing 0.01 *M* citrate, and the naked oocyte thus obtained was compared for RNA content with oocyte with the intact follicular epithelium. No appreciable difference was found between the two series.

Further, the estimation of RNA in the operatively separated nucleus and cytoplasm of the grown oocyte was carried out in a preliminary series and more than 98% of total RNA-P was recovered in the cytoplasmic fraction. This again seems to show that despite its apparent lack of basophilia, the cytoplasm of the grown oocyte does contain an appreciable amount of RNA.

Quantitative measurement of total protein-N was undertaken by the method of Levy and Palmer (*17*) on the "Schneider residue." As shown in Fig. 1, the curve of total protein-N/oocyte rises approximately linearly with the increase of cell volume. If we compare the curve of protein-N with that of RNA-P no simple correlation seems to exist. RNA increase per cell stops before the maximum amount of protein is accumulated. Logarithmic treatment of the data indicates that the rate of increase of RNA per cell is

[3] Chilled alcohol was chosen because of the convenience for tearing the oocytes from the ovarian tissue. The curve obtained from several experiments in which fresh oocytes were fixed and homogenized in cold 10% TCA showed no essential difference from that of alcohol-treated material. The method of differential estimation of RNA and DNA of Ogur and Rosen (*13*) was not suited to our material owing to incomplete separation of the two substances.

[4] In his recent publication, which was received after the completion of this paper, Steinert (*16*) pointed out that RNA in the oocyte of *Rana fusca* increases during its growth.

lower than that of protein accumulation throughout the growth period and that it has a tendency to reduce its value in the later phase of this period.

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## Filter Paper Electrophoresis of Avian Serum Proteins[1]


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The serum proteins of the fowl have been studied by the classical Tiselius' method (*1, 2*), but not, so far as we are aware, by the method of filter paper electrophoresis described by Durrum (*3*) and by Cremer and Tiselius (*4*). The present communication describes preliminary results of studies of fowl sera by filter paper electrophoresis, including studies on the highly lipemic sera of laying and estrogenized birds. Kunkel and Slater (*5*) have pointed out that filter paper electrophoresis may have special advantages for studying lipoprotein components of serum.

The technique was essentially that of Durrum (*3*), as modified by Flynn and De Mayo (*6*). Strips of Whatman 3 MM paper 2½ × 16 in. were used. The Veronal buffer (pH 8.6, ionic strength 0.05) was allowed to rise from both ends of the paper by capillarity until a narrow transverse zone near the anodic end remained unwetted. From 0.1 to 0.2 ml of serum was applied by micropipet to the paper along this zone. The advancing fronts of the buffer were then allowed to come together along the line of application of the serum and before the serum could dry on the paper. This avoided undue diffusion of the proteins, a difficulty which may be accentuated when relatively large amounts of serum (e.g., 0.1–0.2 ml) are applied directly to fully wetted paper strips.

The potential was maintained at 100 v, giving a total current of 8.5 ma when there were 5 strips in the chamber. This current would appear to be smaller than that used by most other workers. A low current reduces trouble from heating and from flow of the buffer. Some (*7, 8*) have described movement of the γ globulin fraction of serum in the opposite direction to the other fractions relative to the place of application. This apparently anomalous movement, due to electroendosomis, is probably accentuated by high currents; it has frequently been absent in this laboratory when using low currents. Control of evaporation and temperature by keeping the papers immersed in a bath of nonaqueous solvent (*4*) was considered inadvisable for studies involving lipoproteins, especially those of the sera of laying or estrogenized fowl. Electrophoresis was usually continued for 24 hr or occasionally longer. Under these conditions the fastest moving major component (the albumin) moved 15–16.5 cm from the zone of application. The drying, staining, and quantitative determination of the dyestuff were performed as described by Flynn and De Mayo (*6*) using bromophenol blue as stain.[3] Lipids were located by exposing the papers to the vapor from a solution of 1% osmic acid in 1% chromic acid. Substances positive to the Liebermann-Burchard reaction were located by immersing papers for a few moments in a reagent made of 10 parts chloroform, 10 parts acetic anhydride, and 1 part concentrated sulfuric acid, and then laying the papers on a glass

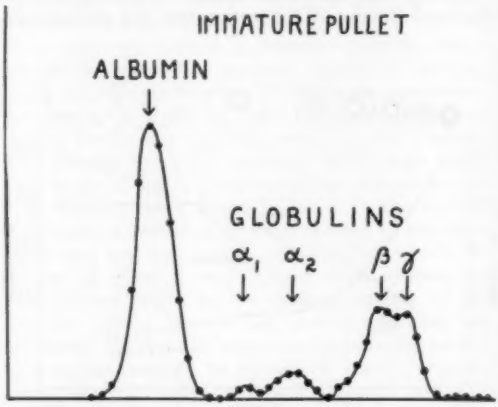



FIG. 1. Quantitative data for paper electrophoresis of avian sera. Vertical axis is optical density. Papers cut in 5-mm transverse strips. A. Immature cockerel, untreated. B. Immature pullet, untreated. C. Turkey hen, nonlaying. D. Normal human.

[1] The project of which this work forms a part is supported by a Grant-in-Aid of Research to the senior author from the National Research Council of Canada.

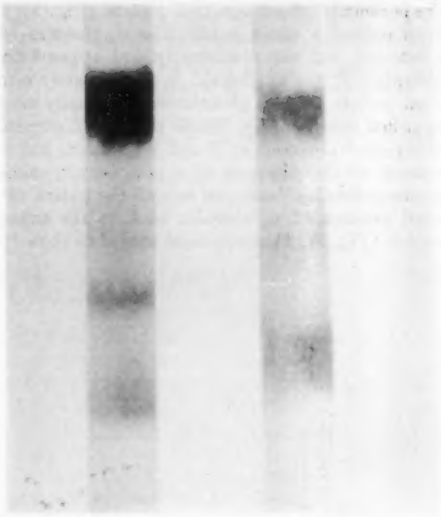
[2] We wish to thank J. H. Quastel and S. F. Van Straten of the Montreal General Hospital Special Research Institute for helpful advice, and W. F. Oliver, Department of Physics, Macdonald College for help with the tracer studies and construction of apparatus. The hormones were generously donated by Messrs. Schering, Ltd., Montreal.

[3] *Added in proof.* In our experience, the staining of protein with this dye, and with naphthalene black, was confined to the superficial layers of the paper.

plate. (This technique was somewhat rudimentary, but demonstrated the practicability of using the Liebermann-Burchard reaction for direct examination of the papers.)

Typical results for turkey, fowl, and human sera are shown in Fig. 1. The data show that the sera of the sexually inactive female fowl and turkey yielded at least 5 distinct protein fractions. The disposition and relative intensity of these 5 fractions bore a close similarity to those recorded for human serum (*9*) and for other vertebrate sera (*7*). Accordingly, we have designated these fractions in order of decreasing mobility as albumin, $\alpha_1$ globulin, $\alpha_2$ globulin, β globulin, and γ globulin by analogy with human serum fractions.

The following points of similarity and contrast between human and fowl sera are noteworthy (Fig. 1): (*a*) The fast-moving albumin fraction had the deepest stain in turkey, fowl, and human sera. (*b*) The $\alpha_1$ globulin fraction gave the faintest stain in all three species. The band is particularly weak with sera from the sexually inactive fowl and may be difficult to distinguish if staining is not sufficiently prolonged. The relative mobility of the $\alpha_1$ fraction appeared to be more variable than that of the other globulin fractions. (*c*) The γ globulin fraction was next to the albumin fraction in intensity of staining in all three species, but moved relatively faster in the avian sera than in human serum. The β globulin fraction stained almost as deeply as the γ globulin fraction, and moved relatively slower in the avian sera than in human serum. (*d*) The $\alpha_2$ globulin fraction stained more deeply than the $\alpha_1$ fraction in all three species, but considerably less deeply than the β globulin. β Globulin appeared to move more slowly relative to the albumin in the avian sera than in human serum.



A B

FIG. 2. Comparison of human and immature pullet sera. A. Human: albumin, β globulin and γ globulin clearly visible; $\alpha_2$ globulin is very faint on photograph. B. Immature pullet: albumin clearly visible, β and globulin so close together that separation is not apparent on photograph; $\alpha_2$ globulin very faint on photograph.

These observations on relative mobilities are summarized in Table 1.

Several wide papers were run in order to compare the mobilities of the avian serum albumins and human serum albumin. The average results are presented in Table 2.

These observations on mobilities suggest that: (*a*) Sera of male or sexually inactive fowl and turkeys yield 5 protein fractions which may be considered analogous to those of human serum. (*b*) The albumin fractions of human, turkey, and fowl sera move at similar speeds. Fowl serum albumin is probably a little slower than human albumin, but the difference is slight. (*c*) The difference in mobility between β and γ globulins is greater in human serum than in male or sexually immature fowl sera (Fig. 2). (*d*) The $\alpha_2$ globulin of fowl serum is slower relative to the albumin than in human serum (Fig. 2).

Osmic acid staining demonstrated that some lipid accompanies the albumin in all sera studied, but we have not detected lipid in association with the globulins with any certainty in immature pullets or cockerels. Durrum *et al.* (*9*) have recorded the presence of lipid along with the albumin of human serum, and also in a region extending from the middle of the $\alpha_2$ globulin band to the middle of the β globulin band in lipemic human serum.

TABLE 1

MOBILITIES OF VARIOUS PROTEIN FRACTIONS OF HUMAN AND AVIAN SERA RELATIVE TO MOBILITIES OF ALBUMIN FRACTIONS*

| | Cockerels | Pullets, sexually immature | Turkey hens, inactive | Human | Human (*9*) |
|---|---|---|---|---|---|
| Albumin | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $\alpha_1$ globulin | 0.86 | 0.73 | 0.76 | 0.87 | 0.83 |
| $\alpha_2$ globulin | 0.60 | 0.59 | 0.65 | 0.70 | 0.66 |
| β globulin | 0.36 | 0.36 | 0.46 | 0.47 | 0.55 |
| γ globulin | 0.30 | 0.27 | 0.31 | 0.17 | 0.22 |

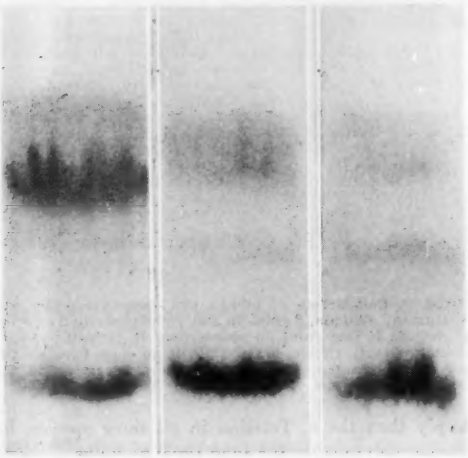
* Running time, 40 to 41 hr; Veronal buffer, pH 8.6, ionic strength 0.05.

TABLE 2

MOBILITIES OF ALBUMIN FRACTIONS OF AVIAN SERA RELATIVE TO MOBILITY OF HUMAN SERUM ALBUMIN

| Source of albumin | Relative mobility |
|---|---|
| Human | 1.00 |
| Goose | 1.00 |
| Cockerel, mature | 0.96 |
| Pullet, sexually immature | 0.98 |
| Hen, laying | 0.98 |
| Turkey, inactive male | 0.94 |

Experiments on estrogenized pullets (Fig. 3) have shown normal albumin bands. The $\alpha_1$ globulin could be detected, but not consistently and appears to be overlapped by the $\alpha_2$ globulin in more heavily estrogenized pullets. The $\alpha_2$ globulin could usually be distinguished satisfactorily. Bands could be discerned in the expected positions of $\beta$ and $\gamma$ globulin, but were obscured by the presence of a new heavily staining fraction extending from just behind the patent or expected position of $\alpha_2$ globulin back to the expected $\beta$ region (Fig. 3). This new band tended to show "tailing," and this was more severe the more heavily the bird had been estrogenized. Kunkle and Slater (*5*) have associated tailing with the presence of neutral fat. The new region also stained rather deeply with osmic acid and gave a positive Liebermann-Burchard reaction. This lipid-staining region included a narrow zone visible as a yellowish band on the unstained paper and exhibited a blue fluorescence in screened UV light. Kunkle and Slater (*5*) have described a somewhat similar orange band in lipemic human sera.



A B C

FIG. 3. A. Normal immature pullet. B. Immature pullet, androgenized. C. Immature pullet, estrogenized. Albumin, $\alpha_2$ globulin, $\beta$ globulin, and $\gamma$ globulin visible in A and B. Note appearance of new protein zone in C, where the new fraction obscures the $\beta$ globulin: this zone displayed tailing and stained deeply with osmic acid. The $\alpha_1$ globulin is not visible.

The new zone associated with lipid was probably in large part due to serum "lipovitellin" and we have designated it as "presumptive lipovitellin" (PLV). Attempts to resolve this PLV band by electrophoresis were based on the use of buffer made up with 20% methanol instead of water. Using this buffer, sera from cockerels or normal immature pullets gave a pattern similar to the normal pattern using the aqueous buffer, but the papers with sera from estrogenized immature pullets showed a distinct new band near the starting line (Fig. 4). This band gave practically no stain with osmic acid, although lipid was still present in the PLV position. Studies using injections of phosphate tagged with $P^{32}$ revealed a high count in the new slow-moving band obtained by use of the methanolic buffer. This high count was not altered appreciably by extraction with Bloor's mixture, whereas a somewhat lower count in the lipid-staining region was almost entirely removed by extraction with Bloor's mixture. It is possible that this new fraction obtained by the use of methanolic buffer represents a phosphoprotein component separated from phospholipid.

A B

FIG. 4. Electrophoresis using Veronal buffer pH 8.6 made up with 20% methanol. A. Immature pullet, untreated. B. Immature pullet, estrogenized. Note that there is a fraction in about the same position as the presumptive phosphovitellin in Fig. 1B, but that the methanolic buffer has resulted in appearance of a further new slow-moving fraction. The band in the PLV position was associated with heavy lipid staining whereas the new slow-moving band was not. An $\alpha_2$ band in paper B was present, but does not show on the photograph.

From the standpoint of technique, it is noteworthy that the intense staining of certain bands on the papers obtained from sera of estrogenized birds may have been due in part to staining of lipid, or to important modifications of the staining properties of the protein due to the presence of lipid. Papers were run in duplicate with normal immature pullet serum and with serum from estrogenized immature pullets. One paper of each pair was extracted with Bloor's mixture before staining. The bands on the paper from normal serum appeared to stain with much the same relative intensities whether extracted or not. The unextracted paper from the estrogenized pullet stained deeply in the fashion typical of such sera, but relative intensities of the bands appeared to have been altered on the papers extracted before staining. It would appear that the interpretation of colorimetric data on dye extracted from stained papers in terms of protein may call for caution when lipemic avian sera are being studied, and further studies on this point are in progress.

A more nearly complete account of our experiments will be published elsewhere.

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Manuscript received January 30, 1953.

# *Book Reviews*

***Plant Anatomy.*** Katherine Esau. New York: Wiley; London: Chapman & Hall, 1953. 735 pp. Illus. + plates. $9.00.

Those who have followed Dr. Esau's research papers and scholarly reviews during the past two decades and have enjoyed her magnificent Kodachrome photomicrographs at scientific meetings have eagerly awaited the appearance of *Plant Anatomy.* This volume should find its way into every up-to-date botanical library, as it will undoubtedly serve for many years as the standard reference on the structure of seed plants.

Although the arrangement of the subject matter follows a conventional sequence—cells, tissues, organs (stems, leaf, root, and reproductive structures)—with topical organization patterned after that of Dr. A. S. Foster in his *Practical Plant Anatomy,* the material has been meticulously reworked from a refreshingly dynamic viewpoint in line with the author's research interest in developmental anatomy. Special mention might be made of the sections on the chemistry and structure of the cell wall, on the meristems, and on the longitudinal course of differentiation in stems, and the final chapters on the flower, fruit, and seed. Here and elsewhere, the results of recent research have been critically reviewed.

Outstanding features of the book are (1) the concisely written introductory statements at the beginning of each chapter in which concepts are outlined and terms defined, (2) the extensive, carefully selected, up-to-the-minute bibliographies on each topic treated, and (3) the superb illustrations. It is somewhat unfortunate that the cost of book manufacture has necessitated grouping all the halftones in plates immediately following the text. This does not seriously impair the usefulness of the volume, however, but it does bring together in a concentrated dose of 90 pages the finest series of photomicrographs of plant structure yet assembled. The majority of these are the author's original photographs of her own preparations or of slides obtained from others. The text figures are also of the highest quality, most of them having been drawn by the author from original material or from published photomicrographs.

To botanists confronted with the problem of selecting a textbook for use in an introductory course in the structure of higher plants, this book poses a difficult problem. On the one hand, the excellence of its coverage and approach points up the serious shortcomings of available texts in the field; on the other, the advanced and comprehensive nature of the book will probably make it difficult reading even for the graduate student. We badly need a moderately priced, illustrated text suitable for the undergraduate, that presents the structure of vascular plants from a dynamic point of view with emphasis on the developmental and physiological aspects of the subject. This reviewer hopes that Dr. Esau can be persuaded to turn her masterly hand to such an assignment.

Richard H. Goodwin
*Department of Botany, Connecticut College*

***Clinical Allergy.*** French K. Hansel. St. Louis, Mo.: Mosby, 1953. 1005 pp. Illus. $17.50.

This volume upon a complex and rapidly expanding subject has been prepared for the student and the general practitioner, as well as for the interested layman. It embraces the various phases of clinical allergy which involve the respiratory, cutaneous, intestinal, neural, and vascular systems. There are presented not only the ideas of the author but also, without bias, those of many other writers and investigators. Consequently, the lists of references which are placed at the close of the chapters are unusually complete and comprehensive.

The first four chapters are devoted to the terminology and basic considerations of allergic conditions; the next seven to discussions of the multitude of etiologic factors of consequence in allergic diseases. One chapter contains much detailed information on the hay fever-producing pollens as found in the different seasons and in the various parts of the United States. This information would have been more immediately accessible to the reader had it been included in tabular form as well, assembled according to seasonal occurrence and sectional distribution.

Adequate space is devoted to various specific and nonspecific diagnostic and treatment procedures as applied to the several clinical forms of allergy. Less than two pages, however, are devoted to the preparation of the allergenic extracts of such vital impor-

tance in diagnosis and treatment. The various drugs of value in allergic conditions are considered, including the newer agents, the antihistaminic drugs and the steroid hormones. Allergic conditions in children are separately treated in five chapters and allergic manifestations of the nose, throat, paranasal sinuses, and ear have been presented in an equal number of chapters.

In *Clinical Allergy* Hansel has presented the result of many hours of study and of effort. Although in a subject such as allergy there are many ideas, concepts, and procedures not yet uniformly acceptable to those practicing in this field, the author has accumulated much valuable information in this volume.

W. C. SPAIN

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***Zoogeography of the Sea.*** Sven Ekman; trans. from Swedish by Elizabeth Palmer. London: Sidgwick and Jackson, 1953. (Distributed by Macmillan, New York.) 417 pp. Illus. $6.50.

Ekman has here extensively revised his *Tiergeographie des Meeres* (1935) and has published it as one of the series of *Textbooks of Animal Biology* under the editorship of H. Munro Fox.

Although the book appears to be intended primarily as a reference work for specialists in oceanography and zoogeography, the general zoologist can hardly fail to find examples and illustrations that will enrich his thoughts and his lectures. Four general types of fauna are dealt with successively and in considerable detail: (1) Shelf fauna (bottom animals living in depths of less than about 200 meters); (2) the deep-sea benthic fauna; (3) plankton and other pelagic life of the upper waters; (4) the bathypelagic fauna. Some 60 per cent of the book is devoted to the first of these groups, and rightly so, since their diversity and their mode of life make them particularly interesting and profitable for zoogeographic study.

Within this broad framework, the subject is subdivided according to geographical regions and temperature zones. Two chapters are devoted to geological and paleontological information, and a careful attempt is made throughout to discuss present-day populations with due regard for their historical background. In the details of regional comparison, Ekman has performed a monumental task in searching the literature and assembling the essential facts about the populations of the various regions. He has drawn upon a list of about 600 references, many of them voluminous expedition reports that even specialists seldom find time to read in their entirety. The information he has gathered represents both synthesis and compilation. A wealth of tabular material is presented on species occurrence, ecological characterizaitons of each region, and other pertinent facts. Graphic and textual descriptions attempt throughout to emphasize small segments of the population that are particularly useful for the exposition of zoogeographic principles.

The amount of factual material that is presented does not make for light nor vastly entertaining reading. It is solid and descriptive throughout. Greater attention to modern zoogeographical theory and to ecological discussions would have made the book more readable although prehaps less enduring. It is characteristic of Ekman that he is able to dismiss the fascinating subject of the origin, history, and general ecology of coral reefs in a mere six pages. Whether this is a criticism or a commendation may be debated among those who have studied the literature.

The concept of temperature zonation is a dominant theme, but nowhere in the book does one find a well-rounded discussion of the physiological background of the subject. More attention to our rapidly growing information on temperature tolerances and to the effects of temperature on respiration, feeding, and reproduction would have illuminated his discussion.

In short, Ekman has chosen to leave out much that is provocative but sometimes misleading. He has not given us any bright new theories, but his excellent descriptive account of marine zoogeography, requiring years of thoughtful and painstaking work, is an invaluable addition to our literature.

GORDON A. RILEY

*The Bingham Oceanographic Laboratory*
*Yale University*

***The Permeability of Natural Membranes.*** Reissue. Hugh Davison and J. F. Danielli. New York: Cambridge Univ. Press, 1952. 365 pp. Illus. $6.00.

It is a distinct pleasure to find this volume, which has been out of print for almost ten years, again available. The authors remark in their new preface that it has not seemed worthwhile to bring out an entirely revised book at this time, and they have merely added bibliographic citations of current literature at the end of each chapter while no changes have been made in the text of the chapters. Such a change is of little help in interpreting to students the very considerable advances that have been made in permeability studies with, for example, the use of radioisotopes, and it is to be hoped that a completely revised work will appear shortly.

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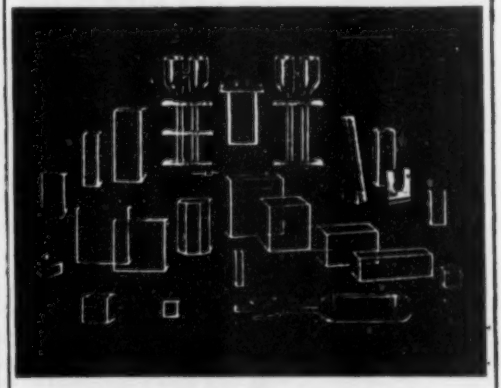

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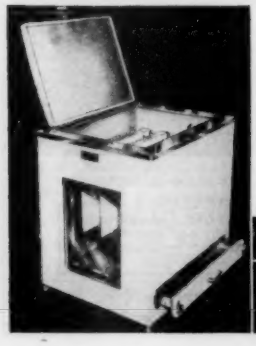

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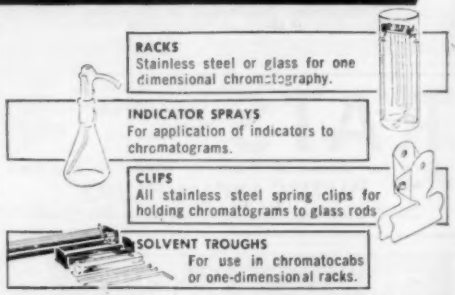
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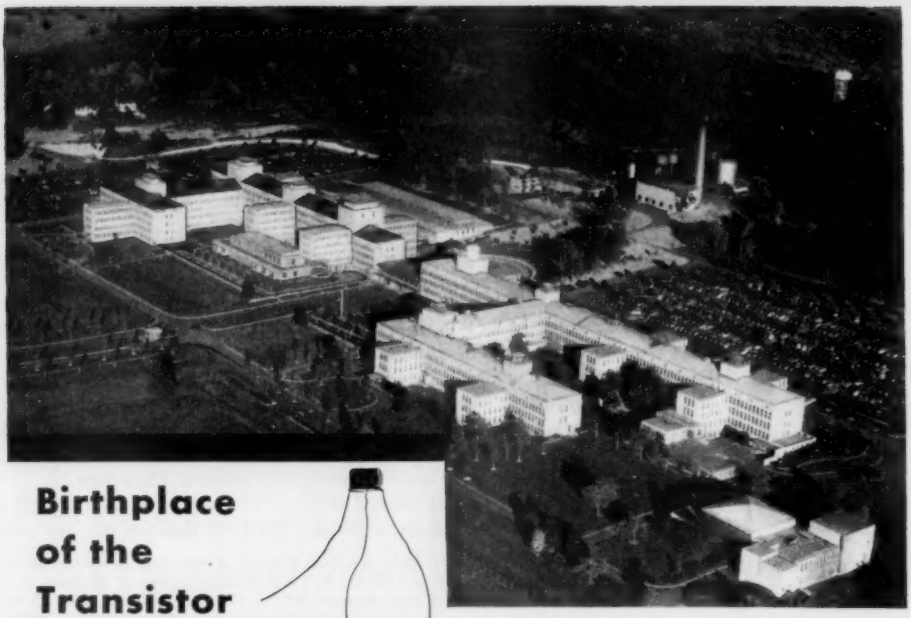

# Birthplace of the Transistor

Bell Telephone Laboratories at Murray Hill, N. J. Other laboratories are in New York City and at Whippany and Holmdel, N. J.

The ***Transistor,*** that revolutionary new electronics device, is a product of *telephone* research. It was conceived, invented and developed at Bell Telephone Laboratories by men in search of ways to improve telephone service. It was announced just five years ago.

The ***Transistor*** can do most of the things that vacuum tubes can do—and others, too—but it is not a vacuum tube. It works on entirely new physical principles. Rugged, simple and tiny, the Transistor uses incredibly small amounts of power—and then only when actually operating.

***Transistors*** promise smaller and cheaper electronics equipment and the spread of electronics where other equipment has not been able to do the job as economically. They are already at work in the Bell System, generating the signals that carry dialed numbers between cities, and selecting the best route for calls through complex switching systems. Engineers see many other possibilities: for example, as voice amplifiers in telephone sets to aid the hard of hearing, and as switches.

Recognizing the tremendous possibilities of the ***Transistor*** in every phase of the electronics industry, the Bell System has made the invention available to 40 other companies. Thus, again, basic research to improve telephony contributes importantly to many other fields of technology as well.

### TRANSISTOR SUMMARY

Basically, a ***Transistor*** is a tiny wafer of germanium with three electrodes, over-all about the size of a coffee bean.

It can amplify signals 100,000 times on much less power than a pocket flashlight requires. This opens the door to its use in smaller telephone exchanges where vacuum tube equipment would be too costly to operate.

Unlike a vacuum tube, the ***Transistor*** has no vacuum and no filament to keep hot. It operates instantly, without "warm-up" delay. The Transistor can also be used as an electric eye and to count electrical pulses.

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